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14. ABSTRACT

Purpose: To determine 1) lung and diaphragm damage at various fractions of inspired oxygen (FIO_2) with and without dopamine following hemorrhagic shock (HS), and 2) the extent of lung damage produced by administering three controlled mechanical ventilator modes following HS. **Design**: A quasi- experimental design using an *in vivo* animal model. **Methods**: Forty percent of the total blood volume was removed from anesthetized rats to elicit HS. Hydrogen peroxide (H_2O_2) and apoptosis were measured in the lung and diaphragm after 60 minutes of administration with the following FIO_2s: 0.21, 0.40, 0.60, 1.00, and with the addition of dopamine (10 µg/kg/min). In another set of experiments, volume control (VC), pressure control (PC), and pressure-regulated volume control (PRVC) MV were administered post HS and lung H_2O_2 and apoptosis were measured. **Sample**: A total of 180 rats were used in experimental protocols. **Analysis**: Differences in lung and diaphragm H_2O_2 and apoptosis were analyzed using analysis of variance (ANOVA) and followed by *post hoc* tests. Significance was defined as $P < 0.05$.
Findings: The optimal FIO_2 to utilize following hemorrhagic shock was 0.40. Dopamine administered intravenously (10 µg/kg/min) scavenged reactive oxygen species (ROS), thus reducing lung and diaphragm damage when higher concentrations of O_2 were used. During HS, the ideal mode of controlled MV was PRVC. MitoSox Red was a potential biomarker for superoxide. **Implications for Military Nursing**: To reduce organ damage following HS, military nurses need to use the optimal O_2 (40%) to prevent excess oxygen molecules to become dangerous free radicals. Dopamine should be administered if higher concentration of O_2 is required. If controlled MV is necessary, a mixed mode of MV such as PRVC should be applied. In addition, using a biomarker such as MitoSox Red would be useful in monitoring the amount of oxidative stress in HS patients.

15. SUBJECT TERMS lung and diaphragm damage, hemorrhagic shock, oxidative stress					
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USU Project Number	N08-012
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III. ABSTRACT

Purpose: To determine 1) lung and diaphragm damage at various fractions of inspired oxygen (FIO_2) with and without dopamine following hemorrhagic shock (HS), and 2) the extent of lung damage produced by administering three controlled mechanical ventilator modes following HS.

Design: A quasi- experimental design using an *in vivo* animal model.

Methods: Forty percent of the total blood volume was removed from anesthetized rats to elicit HS. Hydrogen peroxide (H_2O_2) and apoptosis were measured in the lung and diaphragm after 60 minutes of administration with the following FIO_2s: 0.21, 0.40, 0.60, 1.00, and with the addition of dopamine (10 µg/kg/min). In another set of experiments, volume control (VC), pressure control (PC), and pressure-regulated volume control (PRVC) MV were administered post HS and lung H_2O_2 and apoptosis were measured.

Sample: A total of 180 rats were used in experimental protocols.

Analysis: Differences in lung and diaphragm H_2O_2 and apoptosis were analyzed using analysis of variance (ANOVA) and followed by *post hoc* tests. Significance was defined as $P < 0.05$.

Findings: The optimal FIO_2 to utilize following hemorrhagic shock was 0.40. Dopamine administered intravenously (10 µg/kg/min) scavenged reactive oxygen species (ROS), thus reducing lung and diaphragm damage when higher concentrations of O_2 were used. During HS, the ideal mode of controlled MV was PRVC. MitoSox Red was a potential biomarker for superoxide.

Implications for Military Nursing: To reduce organ damage following HS, military nurses need to use the optimal O_2 (40%) to prevent excess oxygen molecules to become dangerous free radicals. Dopamine should be administered if higher concentration of O_2 is required. If controlled MV is necessary, a mixed mode of MV such as PRVC should be applied. In addition, using a biomarker such as MitoSox Red would be useful in monitoring the amount of oxidative stress in HS patients.

IV. TSNRP RESEARCH PRIORITIES THAT STUDY OR PROJECT ADDRESSES

Primary Priority

Force Health Protection:	☐ Fit and ready force ☐ Deploy with and care for the warrior ☐ Care for all entrusted to our care
Nursing Competencies and Practice:	☐ Patient outcomes ☐ Quality and safety ☒ Translate research into practice/evidence-based practice ☐ Clinical excellence ☐ Knowledge management ☐ Education and training
Leadership, Ethics, and Mentoring:	☐ Health policy ☐ Recruitment and retention ☐ Preparing tomorrow's leaders ☐ Care of the caregiver
	☐

Secondary Priority

Force Health Protection:	☐ Fit and ready force ☒ Deploy with and care for the warrior ☐ Care for all entrusted to our care
Nursing Competencies and Practice:	☐ Patient outcomes ☐ Quality and safety ☐ Translate research into practice/evidence-based practice ☐ Clinical excellence ☐ Knowledge management ☐ Education and training
Leadership, Ethics, and Mentoring:	☐ Health policy ☐ Recruitment and retention ☐ Preparing tomorrow's leaders ☐ Care of the caregiver
Other:	☐

V. PROGRESS TOWARDS ACHIEVEMENT OF SPECIFIC AIMS OF THE STUDY OR PROJECT

a. Findings related to each specific aim, research or study questions, and/or hypothesis:

There were three overall goals in our proposed study. First, to determine the optimal fraction of inspired oxygen (FIO_2) (0.21, 0.40, 0.60, and 1.00) that produces the least lung and diaphragm hydrogen peroxide and deoxyribonucleic acid (DNA) damage (apoptosis) following hemorrhagic shock. Second, to examine the effect of adding dopamine (10 µg/kg/min) at various FIO_2 (0.21, 0.40, 0.60, and 1.00) following hemorrhagic shock. Third, to investigate which control mode of mechanical ventilation (volume, pressure and pressure-regulated volume control) causes the least lung hydrogen peroxide production and apoptosis using the optimal FIO_2. Our goal was to understand lung and diaphragm damage caused by various FIO_2s and control modes of mechanical ventilation following hemorrhagic shock.

Administering 100% oxygen following hemorrhagic shock is a current standard of practice. However, increased production of reactive oxygen species (ROS) may occur at a FIO_2 of 1.00 (100%), because of the increased number of oxygen molecules. Excessive generation of ROS can cause damage to vital organs such as the lungs and diaphragm. Infusing dopamine may help scavenge the additional free radicals produced by 100% oxygen, thus attenuating lung and diaphragm DNA damage but at the same time ensuring increased oxygen supply to vital organs during hemorrhagic shock. Reducing lung and diaphragm hydrogen peroxide production caused by oxygen and control modes of mechanical ventilation may significantly decrease apoptosis, possibly leading to better clinical outcomes for patients in hemorrhagic shock.

Below are our research findings and accomplishments with respect to each specific aim, research question, and hypothesis of our study. We have outlined each of the findings based on the individual aim including the additional addendum items.

AIM 1: To determine the optimal fraction of inspired oxygen (FIO_2) that minimizes nuclear DNA damage in the lung and diaphragm following hemorrhagic shock.

Research question #1: Is hydrogen peroxide production in the lung and diaphragm following 60 minutes of hemorrhagic shock altered at various FIO_2s (0.21, 0.40, 0.60, 1.00)?

Anesthetized rats were administered one of the FIO_2s (0.21, 0.40, 0.60, or 1.00) for 60 minutes following hemorrhagic shock. Hemodynamics (systolic blood pressure, diastolic blood pressure, mean arterial blood pressure, and heart rate) and arterial blood values (pH, $PaCO_2$, HCO_3, base excess, PaO_2, SaO_2, and hemoglobin) were continuously monitored throughout the experiments. Hydrogen peroxide production was determined by fluorescent intensity that was measured by a laser scanning cytometer.

No significant differences in hemodynamics (systolic blood pressure, diastolic blood pressure, mean arterial blood pressure, and heart rate) across the four FIO_2 groups were observed at baseline and shock. After 60 minutes of various FIO_2, diastolic blood pressures and mean arterial blood pressures in three groups (FIO_2 = 0.40, 0.60, and 1.00) were significantly higher than the group breathing FIO_2 at 0.21. In addition, systolic blood pressure in the FIO_2 = 0.60 group was significantly greater than that in the FIO_2 = 0.21 group ($p < 0.05$). The hemodynamic data are presented in Table 1.

Table 1. Hemodynamics for the different fraction of inspired oxygen concentrations

FIO_2	0.21	0.40	0.60	1.00
SBP (mmHg)				
Control	148 ± 4	162 ± 9	157 ± 7	167 ± 9
Shock	97 ± 4	83 ± 3	93 ± 5	94 ± 7
Treatment	93 ± 9	121 ± 14	133 ± 10*	126 ± 14

DBP (mmHg)				
Control	107 ± 5	122 ± 7	115 ± 7	124 ± 7
Shock	43 ± 1	39 ± 2	38 ± 3	46 ± 3
Treatment	38 ± 9	71 ± 13*	76 ± 6*	76 ± 15*
MAP (mmHg)				
Control	127 ± 5	141 ± 7	135 ± 7	143 ± 7
Shock	61 ± 2	54 ± 3	57 ± 3	63 ± 4
Treatment	56 ± 9	94 ± 15*	98 ± 7*	95 ± 16*
HR (b/min)				
Control	357 ± 24	358 ± 10	355 ± 15	334 ± 26
Shock	366 ± 9	326 ± 17	380 ± 11	311 ± 24
Treatment	392 ± 23	379 ± 18	403 ± 19	405 ± 17

Note. Data are expressed as mean ± SEM; n = 6.
SBP = systolic blood pressure; DBP = diastolic blood pressure; MAP = mean arterial pressure; HR = heart rate
* $P < 0.05$ from $FIO_2 = 0.21$.

There were no significant differences in arterial blood values (pH, $PaCO_2$, HCO_3, base excess, PaO_2, SaO_2, and hemoglobin) across the four FIO_2 groups at baseline and shock ($p > 0.05$). After 60 minutes of various FIO_2, PaO_2 significantly increased as the FIO_2 increased. The bicarbonate concentrations in the three groups breathing $FIO_2 = 0.40$, 0.60, or 1.00 were significantly greater than that in the group with $FIO_2 = 0.21$ and accordingly, base excess was significantly less in these three groups. The arterial blood values data are summarized in Table 2.

Table 2. Arterial blood gases for each fraction of inspired oxygen concentration

FIO_2	**0.21**	**0.40**	**0.60**	**1.00**
pH				
Control	7.42 ± 0.02	7.41 ± 0.02	7.40 ± 0.02	7.39 ± 0.02
Shock	7.46 ± 0.03	7.44 ± 0.03	7.47 ± 0.04	7.47 ± 0.04
Treatment	7.24 ± 0.07	7.39 ± 0.04	7.38 ± 0.05	7.31 ± 0.05
$PaCO_2$ (mmHg)				
Control	38 ± 2	41 ± 3	41 ± 1	44 ± 2
Shock	24 ± 3	29 ± 3	24 ± 2	27 ± 3
Treatment	20 ± 2	25 ± 4	27 ± 3	27 ± 5
HCO_3 (mEq/L)				
Control	25 ± 1	26 ± 1	26 ± 1	27 ± 1
Shock	17 ± 1	19 ± 1	17 ± 1	19 ± 1

Treatment	8 ± 1	16 ± 3*	16 ± 2*	15 ± 3*
Beecf				
Control	0.3 ± 0.8	-1.3 ± 1.6	0.8 ± 0.9	1.2 ± 1.0
Shock	-7.3 ± 1.4	-5.0 ± 1.0	-6.3 ± 1.8	-4.3 ± 1.5
Treatment	-19.8 ± 1.5	-9.3 ± 3.1*	-8.8 ± 2.5*	-11.7 ± 4.0
PaO_2 (mmHg)				
Control	80 ± 2	81 ± 4	78 ± 5	77 ± 3
Shock	89 ± 8	86 ± 6	112 ± 8	108 ± 9
Treatment	88 ± 5	196 ± 8*	244 ± 22*	519 ± 21*
O_2 Saturation (%)				
Control	94 ± 1	93 ± 1	95 ± 1	95 ± 1
Shock	97 ± 1	97 ± 1	99 ± 1	99 ± 1
Treatment	97 ± 1	99 ± 1	99 ± 1	99 ± 1
Hgb (g/100 mL)				
Control	12.6 ± 0.6	13.9 ± 0.3	12.5 ± 1.0	14.0 ± 0.9
Shock	7.7 ± 1.0	7.7 ± 0.5	5.8 ± 0.6	8.6 ± 1.0
Treatment	6.0 ± 0.7	6.8 ± 0.5	6.5 ± 0.8	7.0 ± 0.5

Note. Data are expressed as mean ± SEM; n = 6.
$PaCO_2$ = partial pressure carbon dioxide; HCO_3 = bicarbonate; Beecf = base excess; PaO_2 = partial pressure of oxygen; O_2 Saturation = % of oxyhemoglobin; Hgb = hemoglobin
*P < 0.05 from FIO_2 = 0.21.

Data of lung hydrogen peroxide as measured by fluorescent intensity after 60 minutes of various FIO_2 (0.21, 0.40, 0.60, or 1.00) are illustrated in Figure 1. Lung fluorescent intensity was 2.8 X 10^6 for FIO_2 = 0.21, 0.5 X 10^6 for FIO_2 = 0.40, 1.3 X 10^6 for FIO_2 = 0.60, and 3.2 X 10^6 for FIO_2 = 1.00. The differences in lung fluorescent intensity among the four FIO_2 groups were statistically significant. As shown in Figure 1, lung hydrogen peroxide significantly decreased as FIO_2 increased from 0.21 to 0.40 or 0.60 ($p < 0.05$). However, hydrogen peroxide with FIO_2 at 1.00 was not significantly different from that with FIO_2 = 0.21 ($p > 0.05$). The lowest lung hydrogen peroxide production was observed in the group with FIO_2 = 0.40.

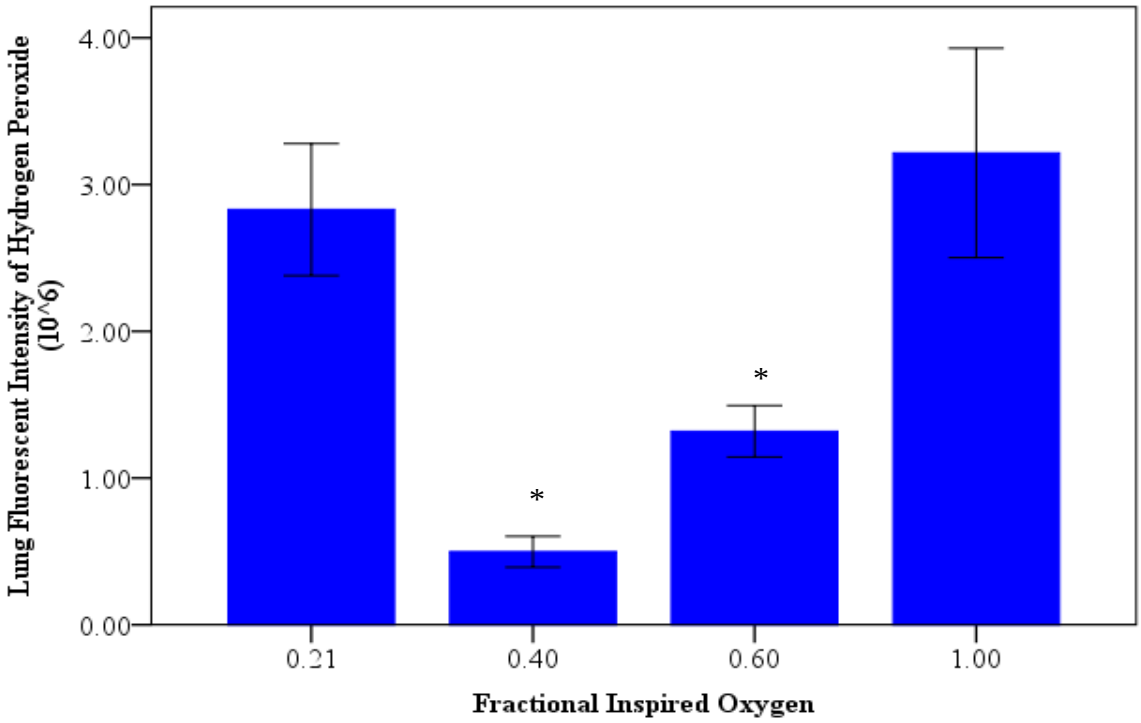


Figure 1. Lung fluorescent intensity of hydrogen peroxide after 60 minutes at various fractional inspired oxygen
Data are presented as mean ± SEM, n = 6.
*P < 0.05 in comparison with FIO_2 = 0.21.

Figure 2 displays data of diaphragm hydrogen peroxide as measured by fluorescent intensity after 60 minutes of various FIO_2 (0.21, 0.40, 0.60, or 1.00). Diaphragm fluorescent intensity was 1.5 X 10^6 for FIO_2 = 0.21, 0.3 X 10^6 for FIO_2 = 0.40, 0.7 X 10^6 for FIO_2 = 0.60, and 1.7 X 10^6 for FIO_2 = 1.00. There were statistically significant differences in diaphragm fluorescent intensity among the four FIO_2 groups. As shown in Figure 2, diaphragm hydrogen peroxide significantly reduced as FIO_2 increased from 0.21 to 0.40 or 0.60 ($p < 0.05$). However, there was a significant increase in diaphragm hydrogen peroxide with FIO_2 at 1.00, in comparison with room air (FIO_2 = 0.21) ($p < 0.05$). Similar to the lung hydrogen peroxide results, the lowest diaphragm hydrogen peroxide production was found in the group with a FIO_2 = 0.40.

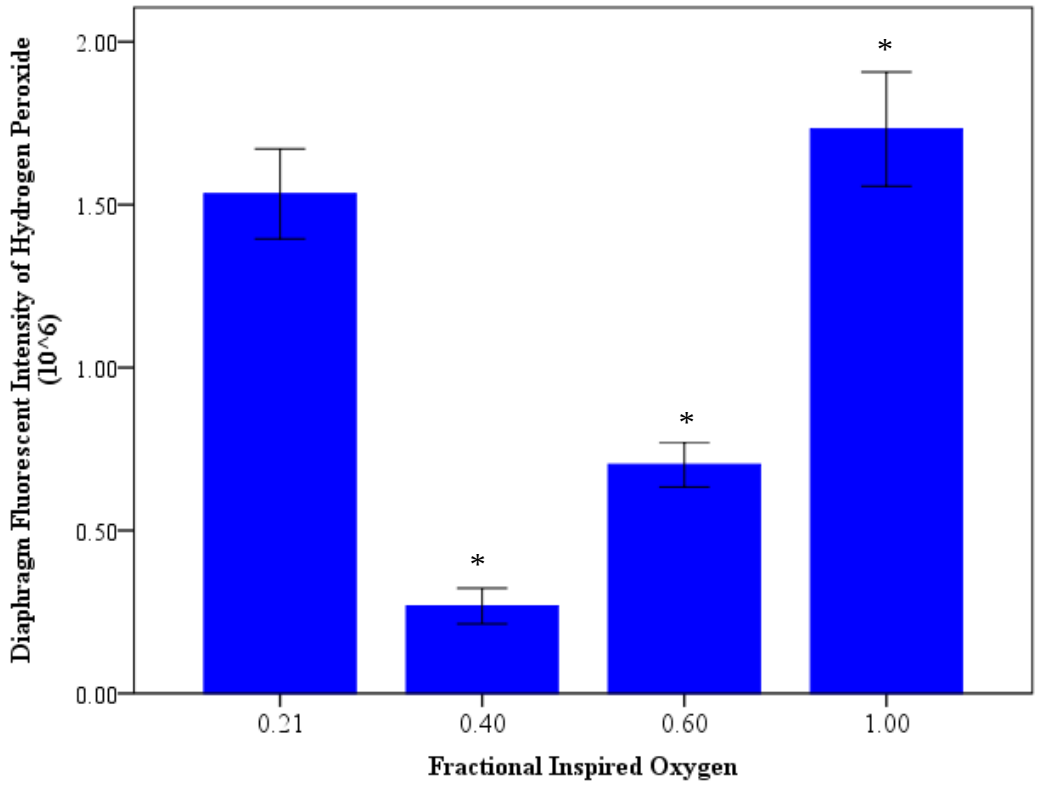


Figure 2. Diaphragm fluorescent intensity of hydrogen peroxide after 60 minutes at various fractional inspired oxygen
Data are presented as mean ± SEM, n = 6.
*P < 0.05 in comparison with FIO_2 = 0.21.

<u>Research question #2</u>: What is the percentage of lung and diaphragm apoptosis (DNA damage) at various FIO_2s (0.21, 0.40, 0.60, 1.00) following 60 minutes of hemorrhagic shock?

Anesthetized rats were given one of the FIO_2s (0.21, 0.40, 0.60, or 1.00) to breathe for 60 minutes following hemorrhagic shock. Hemodynamics (systolic blood pressure, diastolic blood pressure, mean arterial blood pressure, and heart rate) and arterial blood values (pH, $PaCO_2$, HCO_3, base excess, PaO_2, SaO_2, and hemoglobin) were continuously monitored throughout the experiments. Percent apoptosis was determined by differential nuclear dye uptake.

The percentage of lung and diaphragm apoptosis correlated with the hydrogen peroxide production. Percent lung apoptosis following 60 minutes of hemorrhagic shock at various FIO_2 (0.21, 0.40, 0.60, or 1.00) are illustrated in Figure 3. Percent lung apoptosis was highest in the

group breathing room air (FIO_2 = 0.21) with 48.1% and FIO_2 = 1.00 with 57.8%, followed by FIO_2 = 0.60 with 18.7% and lowest in the group breathing a FIO_2 at 0.40 with 2.3%. The differences in percent lung apoptosis among the four FIO_2 groups were statistically significant. As shown in Figure 3, there were significant decreases in percent lung apoptosis as FIO_2 increased from 0.21 to 0.40 ($p \leq 0.001$) or 0.60 ($p = 0.003$). No significant differences in percent lung apoptosis were observed between the group with FIO_2 at 1.00 and the group breathing room air (FIO_2 = 0.21) ($p > 0.05$).

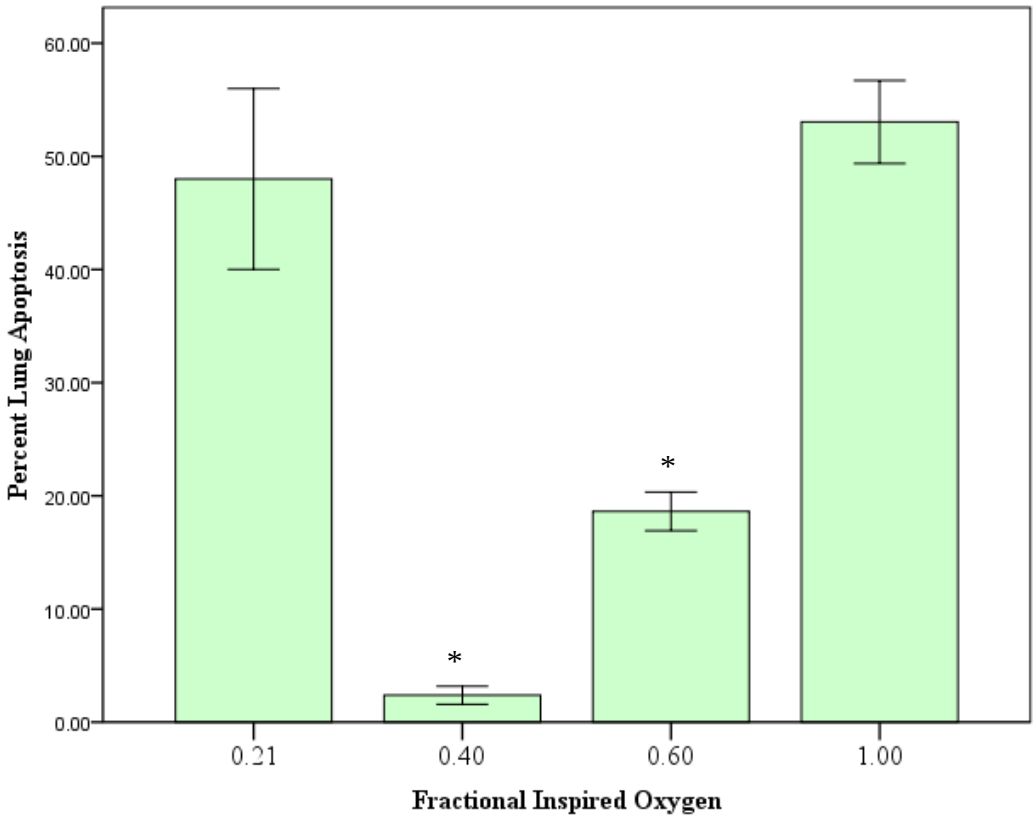


Figure 3. Percent lung apoptosis following 60 minutes hemorrhagic shock at various fractional inspired oxygen
Data are presented as mean ± SEM, n = 8.
*P < 0.05 in comparison with FIO_2 = 0.21.

Figure 4 summarizes percent diaphragm apoptosis following 60 minutes of hemorrhagic shock at various FIO_2 (0.21, 0.40, 0.60, or 1.00). Percent diaphragm apoptosis was 47.9% using a

FIO_2 at 0.21, 1.9% with FIO_2 = 0.40, 15.0% at with FIO_2 = 0.60 and 54.4% with FIO_2 = 1.00. The differences in percent diaphragm apoptosis among the four FIO_2 groups were statistically significant ($p < 0.05$). Illustrated in Figure 4, percent diaphragm apoptosis significantly reduced as FIO_2 increased from 0.21 to 0.40 ($p < 0.05$) or 0.60 ($p < 0.05$). When FIO_2 increased to 1.00, percent diaphragm apoptosis was significantly greater than that in the group with FIO_2 at 0.21 ($p > 0.05$).

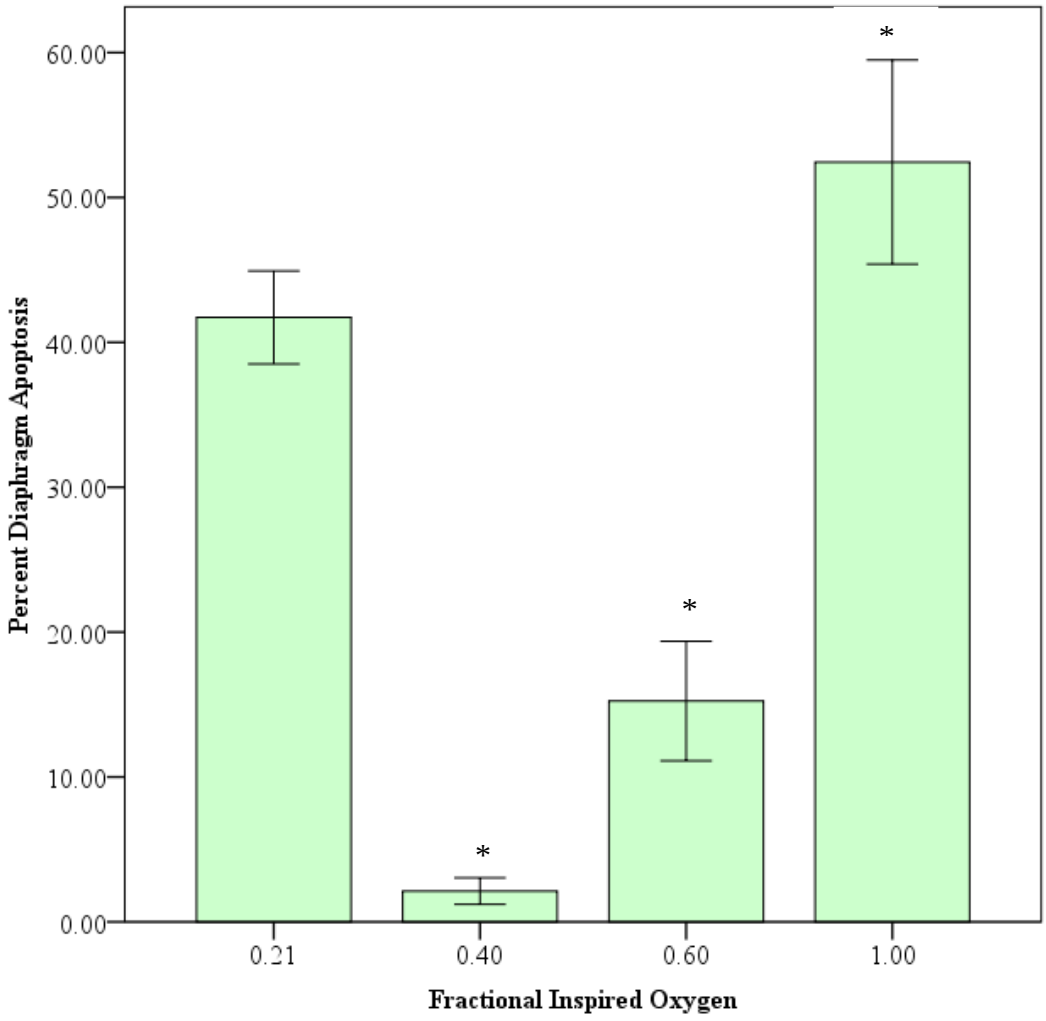


Figure 4. Percent diaphragm apoptosis following 60 minutes hemorrhagic shock at various fractional inspired oxygen
Data are presented as mean ± SEM, n = 8.
*P < 0.05 in comparison with FIO_2 = 0.21.

Summary of findings related to AIM 1: Lung and diaphragm hydrogen peroxide production following 60 minutes hemorrhagic shock with various FIO_2 were greatest with FIO_2s at 0.21 and 1.00, followed by 0.60. The FIO_2 at 0.40 was found to be optimal, as it produced the lowest amount of hydrogen peroxide in both the lung and diaphragm. Nuclear DNA damage in the lungs and diaphragm as measured by percent apoptosis was lowest with the administration of FIO_2 at 0.40 following 60 minutes of hemorrhagic shock. Room air ($FIO_2 = 0.21$) and 100% of oxygen resulted in a significantly higher percent apoptosis, thus a FIO_2 at 0.40 is optimal with minimal lung and diaphragm damage.

AIM 2: To determine the effect of dopamine at the optimal FIO_2 on lung and diaphragm damage following 60 minutes of hemorrhagic shock.

Hypothesis #1: Dopamine attenuates hydrogen peroxide production and apoptosis in the lung and diaphragm following 60 minutes hemorrhagic shock as a function of FIO_2 (0.21, 0.40, 0.60, 1.00).

Anesthetized rats were treated with one of the FIO_2s (0.21, 0.40, 0.60, or 1.00) and an infusion of dopamine (10 µg/kg/min) for 60 minutes following hemorrhagic shock. Hemodynamics (systolic blood pressure, diastolic blood pressure, mean arterial blood pressure, and heart rate) and arterial blood values (pH, $PaCO_2$, HCO_3, base excess, PaO_2, SaO_2, and hemoglobin) were continuously monitored throughout the experiments. Hydrogen peroxide production was determined by fluorescent intensity that was measured by a laser scanning cytometer. Percent apoptosis was measured by differential nuclear dye uptake.

There were no significant differences in hemodynamics (systolic blood pressure, diastolic blood pressure, mean arterial blood pressure, and heart rate) across the four FIO_2 plus dopamine groups at baseline and shock. After 60 minutes treatment with various FIO_2 plus infusion of dopamine, systolic blood pressure in the group administering FIO_2 at 0.60 were significantly decreased, compared to the group breathing room air ($FIO_2 = 0.21$) ($p < 0.05$). No significant

changes in diastolic blood pressure and mean arterial blood pressure were observed as FIO_2 increased. Similarly, heart rates across the four FIO_2 groups were comparable. Table 3 summarizes the hemodynamic data for the four groups with various FIO_2 and administration of dopamine.

Table 3. Hemodynamics for the different fraction of inspired oxygen concentrations plus dopamine

	0.21	0.40	0.60	1.00
SBP (mmHg)				
Control	161 ± 3	153 ± 6	162 ± 9	155 ± 3
Shock	85 ± 4	86 ± 4	93 ± 6	78 ± 6
Treatment	157 ± 10	130 ± 12	122 ± 6*	133 ± 11
DBP (mmHg)				
Control	118 ± 2	111 ± 4	108 ± 6	112 ± 3
Shock	38 ± 1	35 ± 2	36 ± 3	40 ± 2
Treatment	80 ± 9	65 ± 11	70 ± 9	90 ± 11
MAP (mmHg)				
Control	138 ± 2	132 ± 4	135 ± 7	132 ± 3
Shock	55 ± 1	52 ± 2	55 ± 2	54 ± 2
Treatment	104 ± 8	89 ± 11	92 ± 9	109 ± 11
HR (b/min)				
Control	351 ± 11	357 ± 20	314 ± 24	372 ± 18
Shock	324 ± 20	332 ± 17	291 ± 29	311 ± 17
Treatment	402 ± 20	438 ± 31	361 ± 38	396 ± 31

Note. Data are expressed as mean ± SEM; n = 6.
SBP = systolic blood pressure; DBP = diastolic blood pressure; MAP = mean arterial pressure; HR = heart rate
*$P < 0.05$ in comparison with $FIO_2 = 0.21$.

Table 4 presents arterial blood values (pH, $PaCO_2$, HCO_3, base excess, PaO_2, SaO_2, and hemoglobin) at baseline, shock and treatment with different FIO_2 with dopamine infusion. No significant differences in all of the variables were found across the four groups at baseline and shock ($p > 0.05$). After 60 minutes of treatment with FIO_2 greater than 0.21, PaO_2 significantly increased, as compared to that with FIO_2 at 0.21.

Table 4. Arterial blood gases for each fraction of inspired oxygen concentration plus dopamine

FIO_2	0.21	0.40	0.60	1.00
pH				
Control	7.40 ± 0.09	7.43 ± 0.03	7.43 ± 0.04	7.41 ± 0.02
Shock	7.44 ± 0.07	7.43 ± 0.02	7.43 ± 0.05	7.39 ± 0.02
Treatment	7.43 ± 0.02	7.35 ± 0.06	7.39 ± 0.04	7.32 ± 0.05
$PaCO_2$ (mmHg)				
Control	45 ± 1	35 ± 3	43 ± 5	41 ± 2
Shock	32 ± 5	27 ± 2	31 ± 5	34 ± 3
Treatment	25 ± 3	19 ± 2	28 ± 5	36 ± 7
HCO_3 (mEq/L)				
Control	28 ± 1	24 ± 1	27 ± 1	26 ± 1
Shock	21 ± 1	19 ± 1	19 ± 2	21 ± 1
Treatment	16 ± 2	11 ± 2	16 ± 3	19 ± 3
Beecf				
Control	2.0 ± 0.6	-0.3 ± 0.6	2.3 ± 0.6	1.0 ± 0.7
Shock	-3.5 ± 1.7	-5.0 ± 0.7	-5.8 ± 1.3	-4.5 ± 0.4
Treatment	-7.7 ± 1.5	-14.0 ± 3.3	-8.0 ± 3.0	-6.8 ± 3.0
PaO_2 (mmHg)				
Control	72 ± 3	78 ± 5	75 ± 5	72 ± 4
Shock	95 ± 10	84 ± 6	92 ± 4	89 ± 5
Treatment	87 ± 6	181 ± 15*	282 ± 9*	506 ± 27*
O_2 Saturation (%)				
Control	94 ± 1	93 ± 1	95 ± 2	95 ± 1
Shock	97 ± 1	97 ± 1	99 ± 1	99 ± 1
Treatment	97 ± 1	99 ± 1	99 ± 1	99 ± 1
Hgb (g/100 mL)				
Control	13.1 ± 0.4	10.8 ± 0.5	13.1 ± 0.2	13.2 ± 0.5
Shock	8.1 ± 1.0	6.2 ± 0.3	7.3 ± 0.4	7.1 ± 0.7
Treatment	7.1 ± 0.6	5.9 ± 0.3	6.9 ± 0.4	7.1 ± 0.6

Note. Data are expressed as mean ± SEM; n = 6.
$PaCO_2$ = partial pressure carbon dioxide; HCO_3 = bicarbonate; Beecf = base excess; PaO_2 = partial pressure of oxygen; O_2 Saturation = % of oxyhemoglobin; Hgb = hemoglobin
*$P < 0.05$ in comparison with $FIO_2 = 0.21$.

Figure 5 demonstrates the effects of different FIO_2 with dopamine infusion on lung hydrogen peroxide. The greatest lung hydrogen peroxide was found in the group breathing room air (0.87×10^6), followed by FIO_2 at 1.00 (0.57×10^6) and FIO_2 at 0.60 (0.35×10^6). Lung hydrogen peroxide was the lowest in the group using FIO_2 at 0.40 plus dopamine (0.26×10^6).

With administration of dopamine, lung hydrogen peroxide significantly decreased when FIO_2 increased from 0.21 to 0.40, 0.60, or 1.00, as shown in Figure 5.

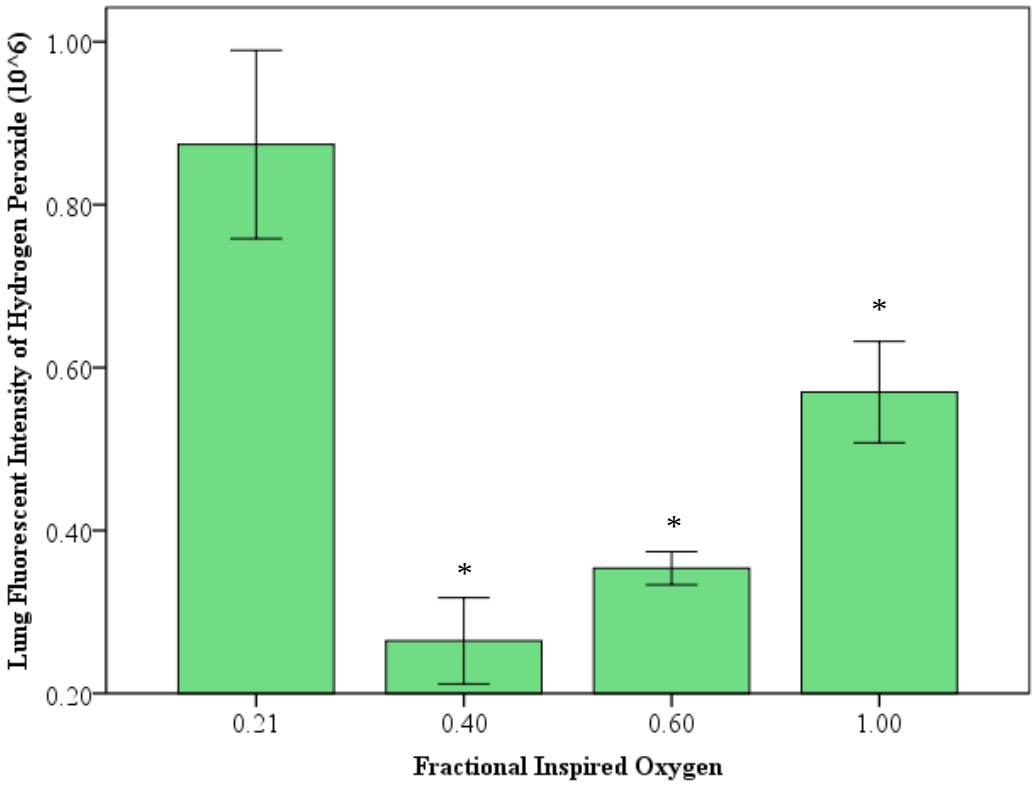


Figure 5. Lung fluorescent intensity of hydrogen peroxide after 60 minutes at various fractional inspired oxygen plus dopamine
Data are presented as mean ± SEM, n = 6.
*P < 0.05 in comparison with FIO_2 = 0.21.

The effect of dopamine on lung hydrogen peroxide was examined by comparing lung hydrogen peroxide data in the FIO_2 only groups and the FIO_2 plus dopamine infusion groups. Results are shown in Figure 6 with blue bars representing the FIO_2 only groups and green bars indicating the FIO_2 plus dopamine infusion groups. Dopamine significantly attenuated lung hydrogen peroxide at FIO_2s of 0.21, 0.60, and 1.00 ($p < 0.05$). There was no significant decrease of lung hydrogen peroxide at FIO_2 of 0.40 with dopamine infusion, as compared to the group with FIO_2 at 0.40 without dopamine.

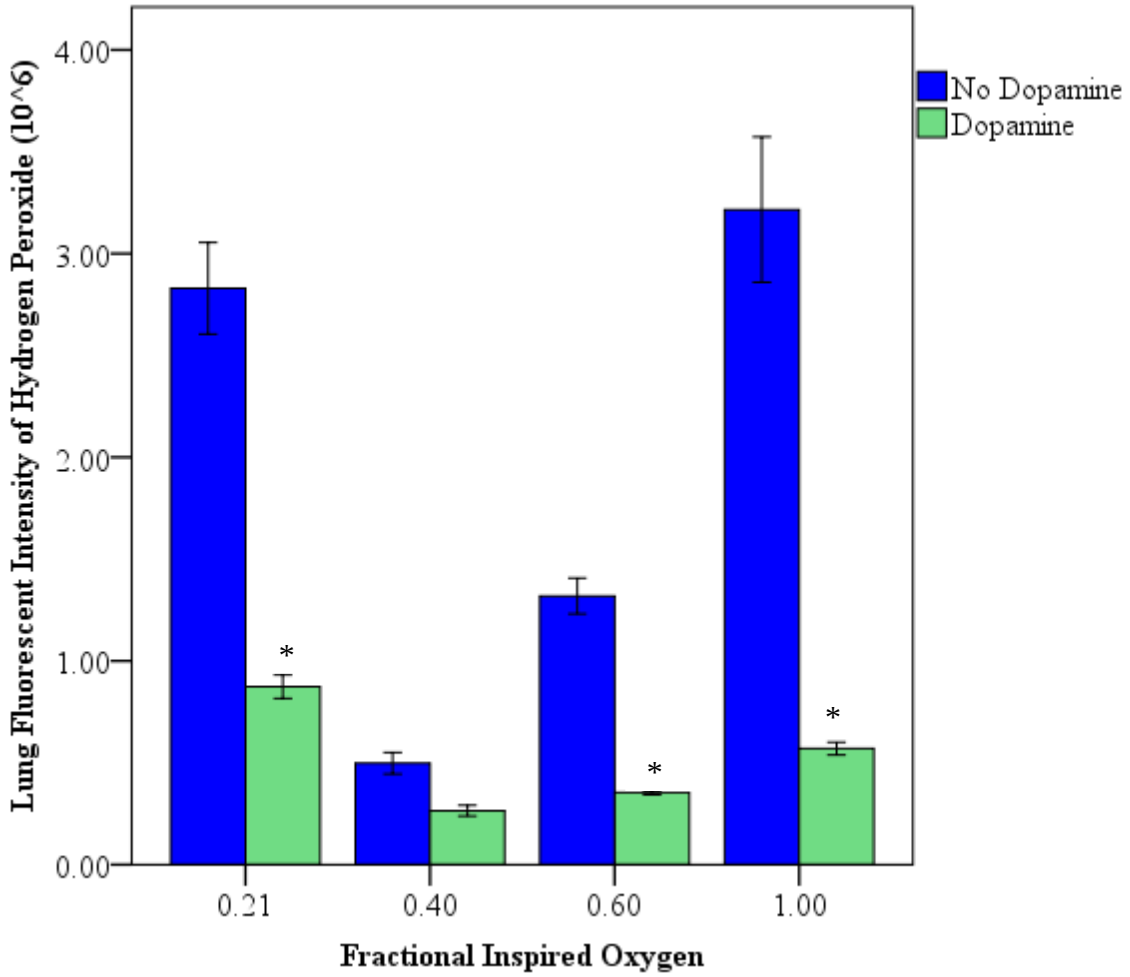


Figure 6. Lung fluorescent intensity of hydrogen peroxide after 60 minutes various fractional inspired oxygen only and plus dopamine
Data are presented as mean ± SEM, n = 6.
*P < 0.05 significantly different between the FIO_2 only and FIO_2 plus dopamine groups.

The effects of different FIO_2 with dopamine infusion on diaphragm hydrogen peroxide are demonstrated in Figure 7. The diaphragm hydrogen peroxide was 0.55 X 10^6 in the group breathing room air (FIO_2 = 0.21), 0.26 X 10^6 with FIO_2 at 0.40, 0.36 X 10^6 with FIO_2 at 0.60, and 0.59 X 10^6 FIO_2 at 1.00. The greatest diaphragm hydrogen peroxide was found in the group with FIO_2 at 1.00 plus dopamine and the group using FIO_2 at 0.40 plus dopamine had the lowest diaphragm hydrogen peroxide. Administration of dopamine significantly decreased diaphragm hydrogen peroxide in the groups with FIO_2 = 0.40 and 0.60, when compared to the group with

FIO_2 = 0.21 (shown in Figure 7). However, there was no significant reduction of diaphragm hydrogen peroxide as FIO_2 increased from 0.21 to 1.00.

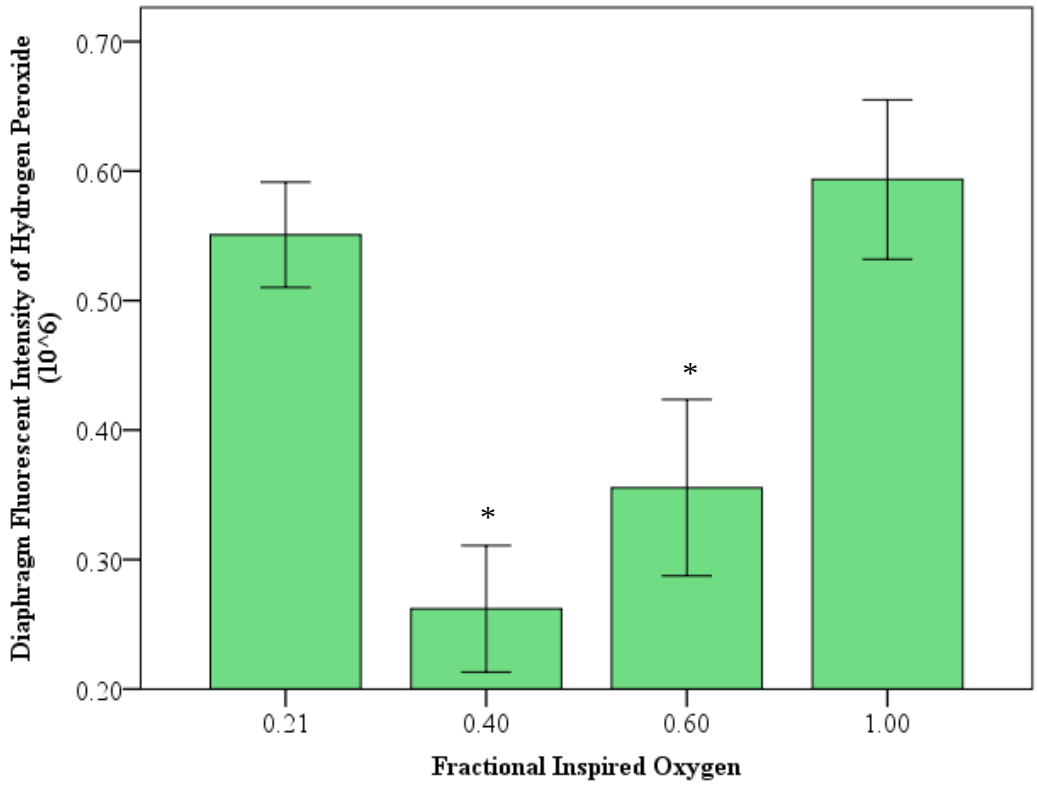


Figure 7. Diaphragm fluorescent intensity of hydrogen peroxide after 60 minutes at various fractional inspired oxygen plus dopamine
Data are presented as mean ± SEM, n = 6.
*P < 0.05 in comparison with FIO_2 = 0.21.

Diaphragm hydrogen peroxide in the FIO_2 only groups and the FIO_2 plus dopamine infusion groups were compared to examine the effect of dopamine on diaphragm hydrogen peroxide. Data are displayed in Figure 8 with blue bars representing the FIO_2 only groups and green bars identifying the FIO_2 plus dopamine infusion groups. Dopamine infusion led to significant decreases in diaphragm hydrogen peroxide at all FIO_2s except 0.40 ($p < 0.05$). Diaphragm hydrogen peroxide at FIO_2 of 0.40 with dopamine infusion was not significantly less than the group with FIO_2 at 0.40 without dopamine.

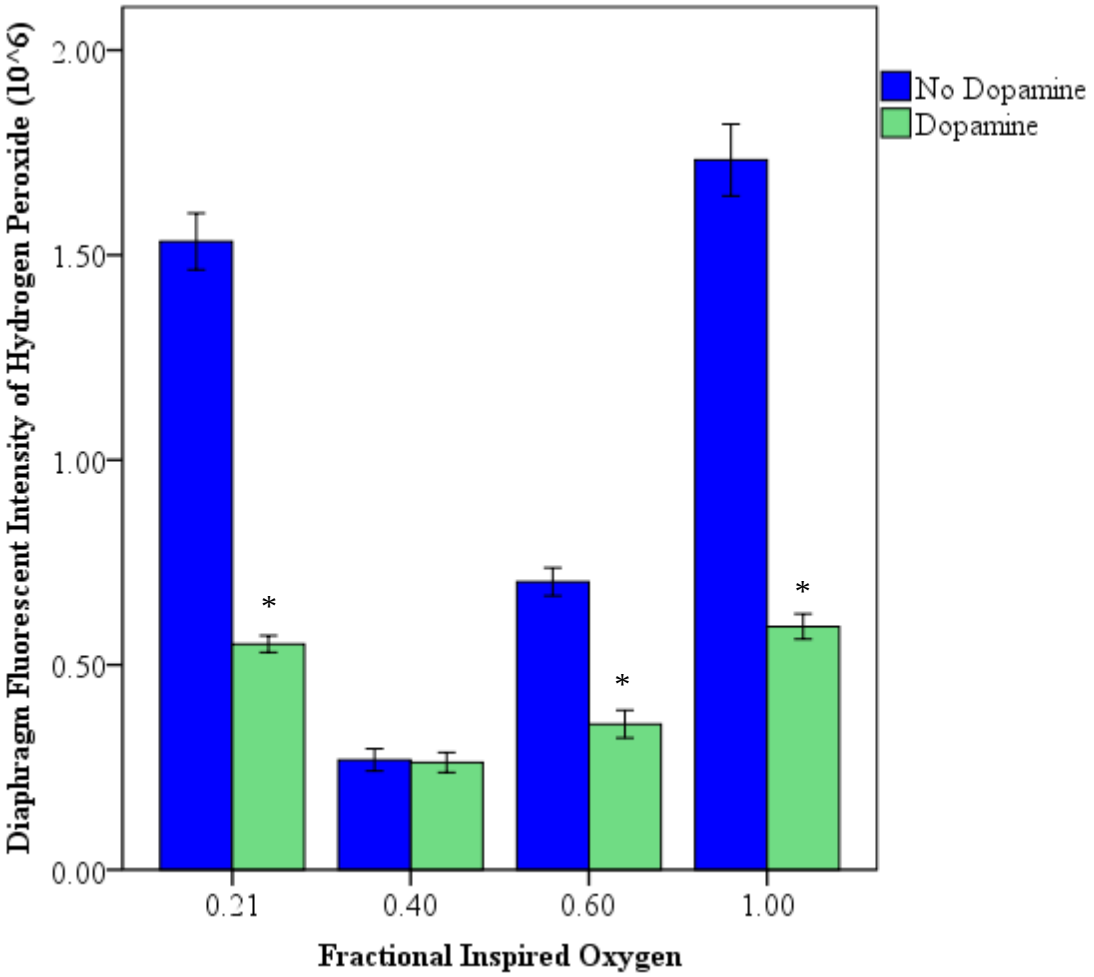


Figure 8. Diaphragm fluorescent intensity of hydrogen peroxide after 60 minutes various fractional inspired oxygen only and plus dopamine
Data are presented as mean ± SEM, n = 6
*P < 0.05 significantly different between the FIO_2 only and FIO_2 plus dopamine groups

Figure 9 shows percent lung apoptosis at various FIO_2 with infusing dopamine. Lung apoptosis with the higher percent occurred with the use of FIO_2s 0.21 (6.5%) and 1.00 (12.9%). A significant increase in percent lung apoptosis was only observed at FIO_2 of 1.00, as increasing FIO_2 from 0.21 while giving dopamine. With administering dopamine, percent lung apoptosis at FIO_2 of 0.21 was not significantly different from FIO_2 at 0.40 ($p \leq 0.117$) and FIO_2 at 0.60 ($p = 0.401$).

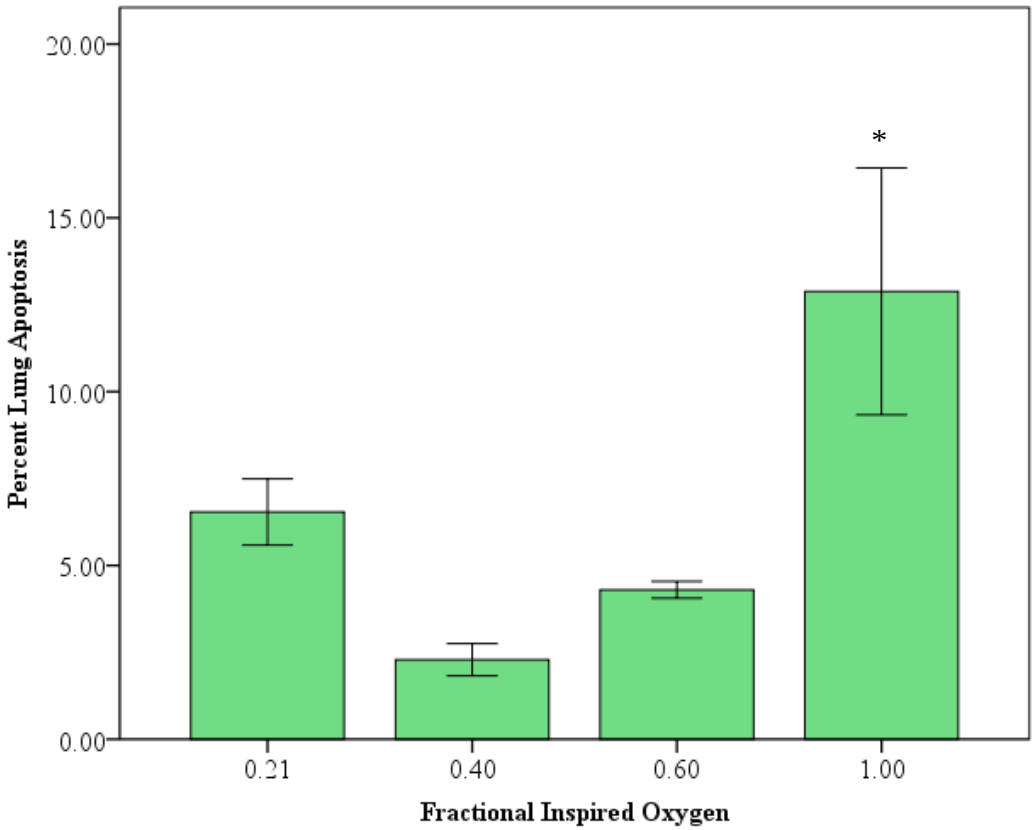


Figure 9. Percent lung apoptosis following 60 minutes hemorrhagic shock at various fractional inspired oxygen plus dopamine
Data are presented as mean ± SEM, n = 8.
*P < 0.05 in comparison with FIO_2 = 0.21.

The effect of dopamine on percent lung apoptosis was evaluated by comparing percent lung apoptosis in the FIO_2 plus dopamine infusion groups to the FIO_2 only groups. Results are summarized in Figure 10 with blue bars identifying the FIO_2 only groups and green bars signifying the FIO_2 with dopamine infusion groups. Dopamine significantly decreases percent lung apoptosis at FIO_2s of 0.21, 0.60, and 1.00 ($p < 0.05$). There was no significant decrease of percent lung apoptosis with FIO_2 at 0.40 plus administering dopamine, when compared to the group with FIO_2 at 0.40 only.

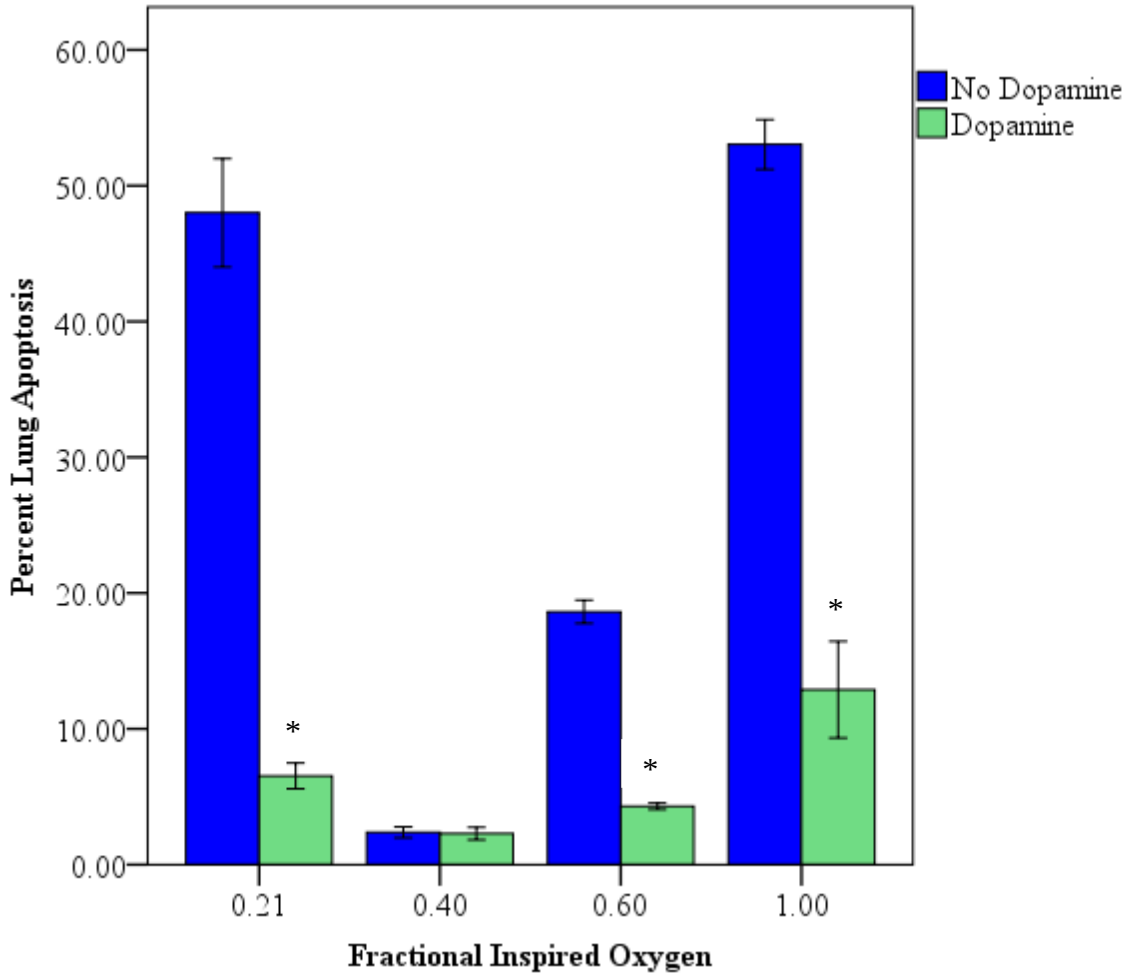


Figure 10. Percent lung apoptosis after 60 minutes various fractional inspired oxygen only and plus dopamine
Data are presented as mean ± SEM, n = 8.
*P < 0.05 significantly different between the FIO_2 only and FIO_2 plus dopamine groups.

Percent diaphragm apoptosis at different FIO_2 plus administering dopamine are illustrated in Figure 11. With infusing dopamine, percent diaphragm apoptosis was 2.9% using a FIO_2 at 0.21, 2.4% at 0.40, 3.2% at 0.60 and 9.9% at 1.00. No significant decreases in percent diaphragm apoptosis were observed at FIO_2 of 0.40 or 0.60 after administering dopamine. However, there was a significant elevation of percent diaphragm apoptosis at FIO_2 of 1.00 ($p < 0.05$), as increasing FIO_2 from 0.21 while giving dopamine.

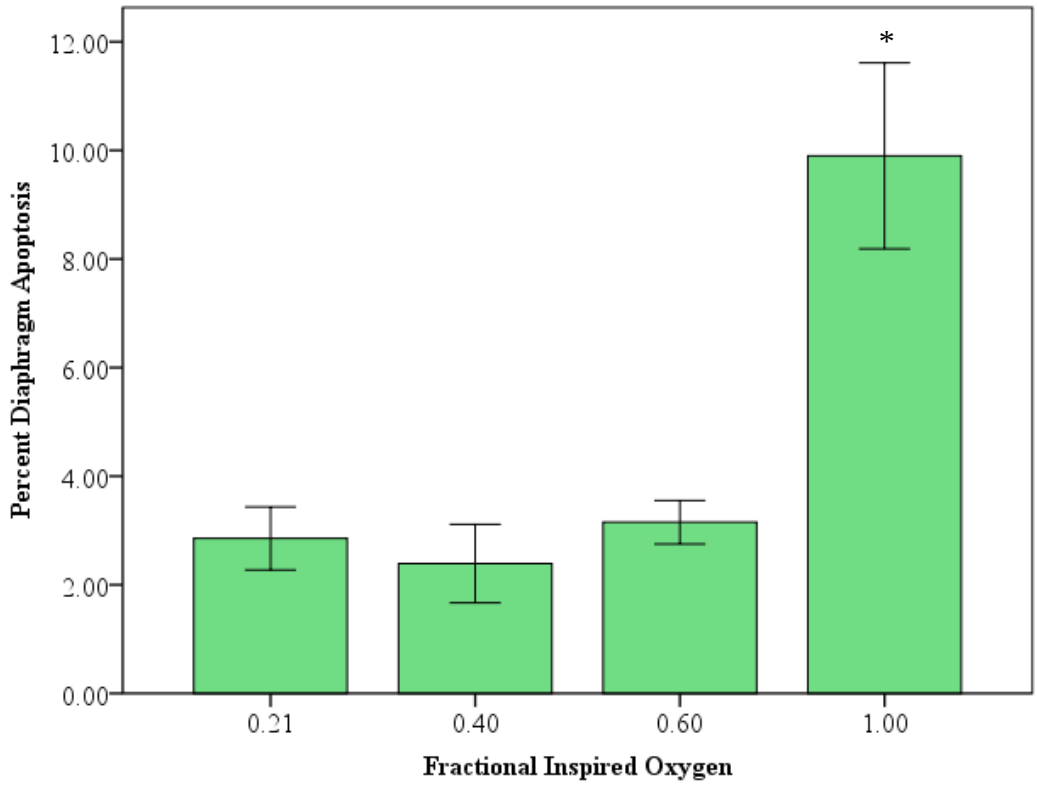


Figure 11. Percent diaphragm apoptosis following 60 minutes hemorrhagic shock at various fractional inspired oxygen plus dopamine

Data are presented as mean ± SEM, n = 8.
*P < 0.05 in comparison with FIO_2 = 0.21.

Figure 12 illustrates the results of comparing percent diaphragm apoptosis in the FIO_2 only groups (blue bar) to the FIO_2 with dopamine groups (green bar). Administering dopamine resulted in significant decreases in diaphragm apoptosis at all FIO_2s except 0.40 ($p < 0.05$). There was no significantly reduction in diaphragm apoptosis at FIO_2 of 0.40 with dopamine infusion.

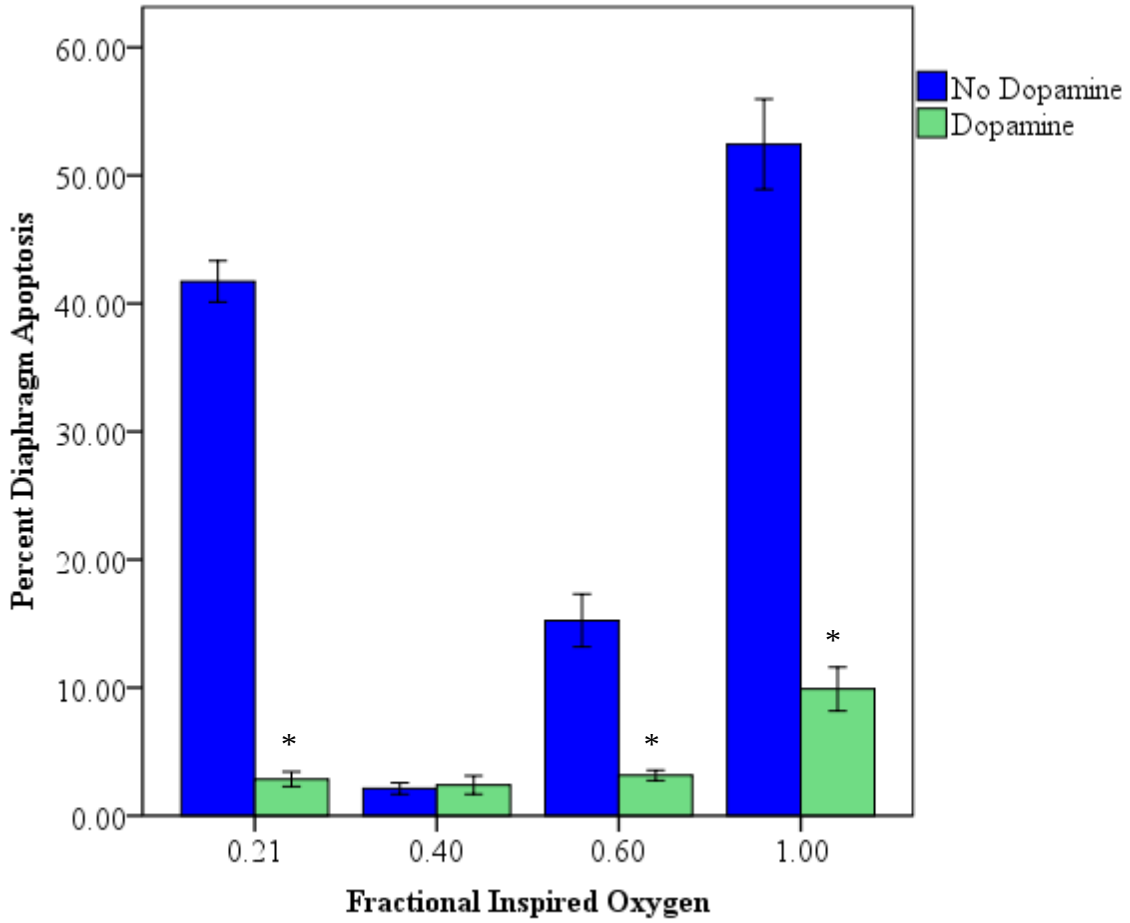


Figure 12. Percent diaphragm apoptosis after 60 minutes at various fractional inspired oxygen only and plus dopamine
Data are presented as mean ± SEM, n = 8.
*P < 0.05 significantly different between the FIO_2 only and FIO_2 plus dopamine groups.

Summary of findings related to AMI 2: Administering dopamine in conjunction with a FIO_2 at 0.21, 0.60, and 1.00 significantly attenuated production of hydrogen peroxide and percentage of apoptosis in the lungs and the diaphragm following 60 minutes of hemorrhagic shock. The amount of lung and diaphragm hydrogen peroxide and percent of apoptosis at a FIO_2 of 0.40 did not significantly decrease with addition of dopamine.

AIM 3: To determine if controlled mechanical ventilation alters lung hydrogen peroxide production and apoptosis following hemorrhagic shock at optimal FIO_2.

Question #1: Does volume control, pressure control and pressure-regulated volume control alter lung hydrogen peroxide production and apoptosis following 60 minutes of hemorrhagic shock?

Anesthetized rats were placed on one of the three controlled mechanical ventilation modes (volume control, pressure control, or pressure-regulated volume control) using a FIO_2 = 0.40 for 60 minutes post hemorrhagic shock. The parameters of controlled mechanical ventilation were set as following: ventilation rate = 80 breaths per minute, positive end-expiratory pressure = 1 cm H_2O, peak inspiratory pressure = 8 cm H_2O, and inspired tidal volume = 4.0 mL.

Hemodynamics (systolic blood pressure, diastolic blood pressure, mean arterial blood pressure, and heart rate), arterial blood values (pH, $PaCO_2$, PaO_2, SaO_2, hematocrit, and hemoglobin), and pulmonary parameters (peak inspiratory pressures, tidal volumes, respiratory rate, and intrathoracic pressures) were continuously monitored throughout the experiments.

Overall, there were no statistically significant differences in hemodynamics, arterial blood values, and pulmonary parameters across the three controlled mechanical ventilation groups at baseline, shock, and treatment, but with a few exceptions. Table 5 and 6 summarize hemodynamics and arterial blood values, respectively. Pulmonary parameters including peak inspiratory pressures, tidal volumes, and respiratory rate are shown in Table 7. Intrathoracic pressures are displayed in Table 8.

Table 5. Blood pressures and heart rates at baseline, hemorrhagic shock, and during 30 and 60 minutes of mechanical ventilation

	VC	PC	PRVC
Baseline			
SBP	170 ± 7	137 ± 11	165 ± 11
DBP	122 ± 5	98 ± 9	118 ± 6
MAP	142 ± 4	117 ± 10	140 ± 8
HR	347 ± 10	352 ± 8	369 ± 11
Shock			
SBP	74 ± 6 †	79 ± 7 †	84 ± 2 †

DBP	38 ± 6 †	38 ± 4 †	41 ± 3 †
MAP	50 ± 6 †	51 ± 6 †	54 ± 2 †
HR	346 ± 10	335 ± 16	363 ± 20
30 Minutes			
SBP	80 ± 6 †	94 ± 11 †	84 ± 2 †
DBP	36 ± 6 †	52 ± 5 †	40 ± 4 †
MAP	50 ± 6 †	68 ± 7 †	54 ± 4 †
HR	326 ± 9	338 ± 13	340 ± 13
60 Minutes			
SBP	88 ± 8 †	104 ± 10	77 ± 8 †
DBP	36 ± 5 †	54 ± 5 † *	31 ± 2 † **
MAP	54 ± 6 †	76 ± 8 †	45 ± 4 † **
HR	306 ± 10 †	332 ± 17	333 ± 10

Note. Data are presented as mean ± SEM, n = 5 – 6.
VC = volume control, PC = pressure control, PRVC = pressure regulated volume control, SBP = systolic blood pressure (mm Hg), DBP = diastolic blood pressure (mm Hg), MAP = mean arterial pressure (mmHg), HR = heart rate (beats/min)
†P < 0.05 in comparison with baseline.
*P < 0.05 in comparison with VC.
**P < 0.05 in comparison with PC.

Table 6. Arterial blood values at baseline, hemorrhagic shock, and following 60 minutes of mechanical ventilation

	VC	PC	PRVC
Baseline			
pH	7.40 ± 0.02	7.33 ± 0.07	7.42 ± 0.02
$PaCO_2$	45 ± 2	44 ± 2	43 ± 2
PaO_2	75 ± 2	77 ± 5	81 ± 7
SaO_2	94 ± 1	94 ± 1	95 ± 1
Hct	42 ± 2	37 ± 4	40 ± 2
Hbg	14.2 ± 0.6	12.4 ±1.2	14 ± 0.6
Shock			
pH	7.41 ± 0.05	7.39 ± 0.05	7.49 ± 0.06
$PaCO_2$	26 ± 2 †	34 ± 4	23 ± 4 †
PaO_2	100 ± 6	96 ± 5	103 ± 12
SaO_2	97 ± 1	98 ± 1 †	100 ± 0 †
Hct	17 ± 0 †	22 ± 3 †	18 ± 2 †
Hbg	5.8 ± 0.3 †	7.6 ± 0.9 †	5.9 ± 0.6 †
60 Minutes			
pH	7.31 ± 0.03	7.39 ± 0.05	7.29 ± 0.07
$PaCO_2$	31 ± 4 †	35 ± 3	34 ± 4

PaO_2	211 ± 10 † ‡	228 ± 10 † ‡	198 ± 15 † ‡
SaO_2	100 ± 0 †	100 ± 0 †	100 ± 0 †
Hct	15 ± 1 †	16 ± 2 †	15 ± 3 †
Hbg	5.3 ± 1.1 †	6.1 ± 1.0 †	5.0 ± 0.9 †

Note. Data are presented as mean ± SEM, n = 4 – 5.
VC = volume control, PC = pressure control, PRVC = pressure regulated volume control, pH = acidity, $PaCO_2$ = arterial carbon dioxide (mm Hg), PaO_2 = arterial oxygen (mmHg), SaO_2 = % oxygen saturation, Hct = hematocrit, Hgb = hemoglobin (gm/100 ml)
†P < 0.05 in comparison with baseline
‡P < 0.05 in comparison with shock

Table 7. Peak inspiratory pressures, tidal volumes, and respiratory rates during three modes of mechanical ventilation

	VC	PC	PRVC
PIP			
1 Min	8.5 ± 0.2	7.7 ± 0.2	8.3 ± 0.4
10 Min	8.3 ± 0.4	7.7 ± 0.2	8.2 ± 0.5
20 Min	8.3 ± 0.3	7.7 ± 0.2	8.4 ± 0.2
30 Min	8.3 ± 0.3	7.8 ± 0.3	7.8 ± 0.5
40 Min	8.7 ± 0.2	7.5 ± 0.2 *	8.0 ± 0.4
50 Min	8.3 ± 0.3	7.7 ± 0.2	8.2 ± 0.4
60 Min	8.6 ± 0.2	7.5 ± 0.2 *	8.0 ± 0.4
Tidal Volume			
1 Min	4.0 ± 0.0	4.1 ± 0.2	4.0 ± 0.0
10 Min	4.0 ± 0.0	3.9 ± 0.1	4.0 ± 0.0
20 Min	4.0 ± 0.0	3.9 ± 0.1	4.0 ± 0.0
30 Min	4.0 ± 0.0	3.8 ± 0.1 *	4.0 ± 0.0 **
40 Min	4.0 ± 0.0	3.8 ± 0.1 *	4.0 ± 0.0 **
50 Min	4.0 ± 0.0	3.6 ± 0.1 *	4.0 ± 0.0 **
60 Min	4.0 ± 0.0	3.8 ± 0.1	4.0 ± 0.0
RR			
1 Min	81 ± 0	80 ± 0	78 ± 2
10 Min	81 ± 0	82 ± 1	80 ± 0
20 Min	81 ± 1	82 ± 2	80 ± 0
30 Min	80 ± 0	80 ± 0	83 ± 1
40 Min	80 ± 0	80 ± 0	83 ± 3
50 Min	80 ± 0	81 ± 1	79 ± 1
60 Min	79 ± 2	80 ± 0	80 ± 0

Note. Data are presented as mean ± SEM, n = 5 - 6

VC = volume control, PC = pressure control, PRVC = pressure regulated volume control, PIP = peak inspiratory pressure (cm H_2O), Tidal Volume (mL), RR = respiratory rate (breaths/min), Min = minutes
*P < 0.05 in comparison with VC
**P < 0.05 in comparison with PC

Table 8. Intrathoracic pressures at baseline, hemorrhagic shock, and during three modes of mechanical ventilation

	VC	PC	PRVC
Inspiration			
Baseline	-3.0 ± 0.2	-2.9 ± 0.2	-2.5 ± 0.2
Shock	-2.7 ± 0.4	-3.5 ± 0.5	-2.9 ± 0.3
10 Min	2.6 ± 0.4 † ‡	2.7 ± 0.3 † ‡	2.8 ± 0.2 † ‡
20 Min	2.6 ± 0.3 † ‡	2.7 ± 0.3 † ‡	2.8 ± 0.3 † ‡
30 Min	2.6 ± 0.3 † ‡	2.6 ± 0.3 † ‡	2.9 ± 0.2 † ‡
40 Min	2.8 ± 0.3 † ‡	2.7 ± 0.2 † ‡	3.1 ± 0.2 † ‡
50 Min	2.8 ± 0.4 † ‡	3.0 ± 0.4 † ‡	3.1 ± 0.2 † ‡
60 Min	3.1 ± 0.4 † ‡	3.0 ± 0.4 † ‡	3.0 ± 0.2 † ‡
Expiration			
Baseline	1.0 ± 0.1	0.9 ± 0.2	1.2 ± 0.2
Shock	0.8 ± 0.2	0.7 ± 0.1	1.1 ± 0.3
10 Min	0.8 ± 0.2	0.8 ± 0.2	0.9 ± 0.3
20 Min	0.9 ± 0.2	0.6 ± 0.1	1.1 ± 0.4
30 Min	0.9 ± 0.2	0.5 ± 0.1	1.0 ± 0.3
40 Min	0.7 ± 0.2	0.8 ± 0.2	1.0 ± 0.2
50 Min	0.4 ± 0.5	0.7 ± 0.3	1.0 ± 0.2
60 Min	0.8 ± 0.3	0.8 ± 0.3	1.0 ± 0.2

Note. Esophageal pressure (cm H_2O) was measured as intrathoracic pressure.
Data are presented as mean ± SEM, n = 6.
VC = volume control, PC = pressure control, PRVC = pressure regulated volume control, Min = minutes
†P < 0.05 in comparison with baseline
‡P < 0.05 in comparison with shock

Significant differences were found in lung hydrogen peroxide production among the three modes of controlled mechanical ventilation ($p < 0.05$). Data are presented as mean ± SEM. Lung hydrogen peroxide measured by fluorescent intensity for volume control was 1.84 X 10^6 ± 0.14, 1.11 X 10^6 ± 0.04 with pressure control and 0.36 X 10^6 ± 0.07 with pressure-regulated volume control. As shown in Figure 13, lung hydrogen peroxide in the volume control group was 66%

higher than the pressure control group and more than 5 times greater than the pressure-regulated volume control group. The pressure-regulated volume control group had the lowest lung hydrogen peroxide production.

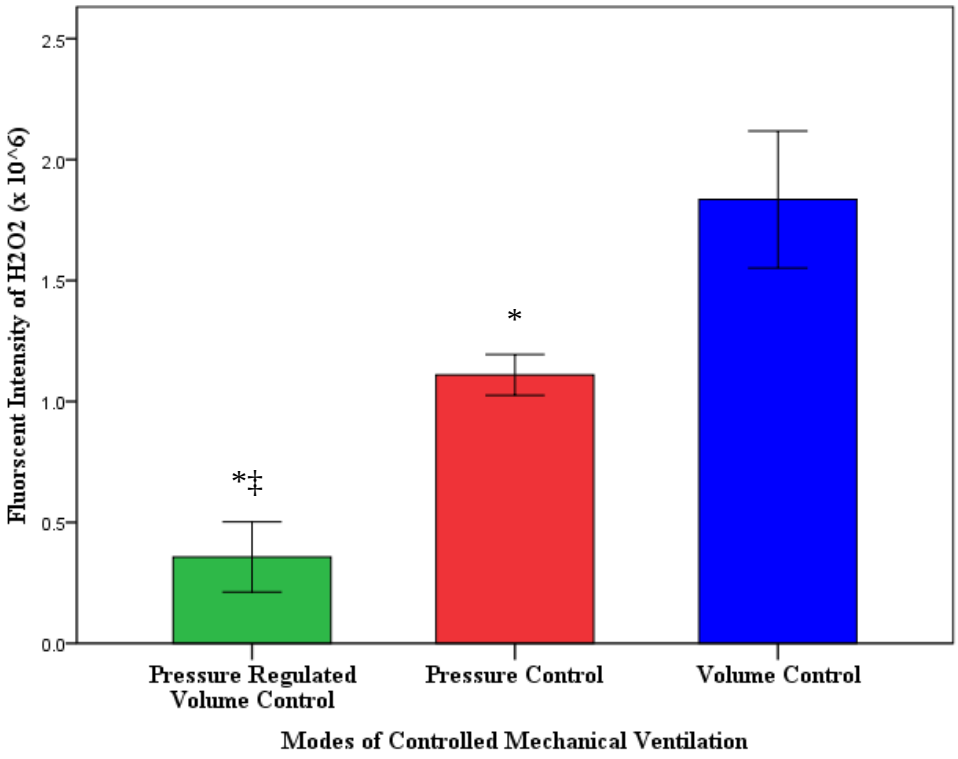


Figure 13.Lung hydrogen peroxide production after 60 minutes of controlled mechanical ventilation during hemorrhagic shock
Data are presented as mean ± SEM, n = 6.
*P < 0.05 in comparison with volume control.
‡P < 0.05 in comparison with pressure control.

Percent lung apoptosis for the three modes of controlled mechanical ventilation are presented in Figure 14. Percent lung apoptosis was 20.9 ± 0.9 for volume control, 12.1 ± 0.6 for pressure control, and 4.5 ± 0.1 for pressure-regulated volume control. Consistent with lung hydrogen peroxide production, the differences of lung apoptosis observed across the three modes of controlled mechanical ventilation were statistically significant ($p < 0.05$). In the volume control group, percent lung apoptosis was 73% higher than the pressure control group and 4 times greater than the pressure-regulated volume control group.

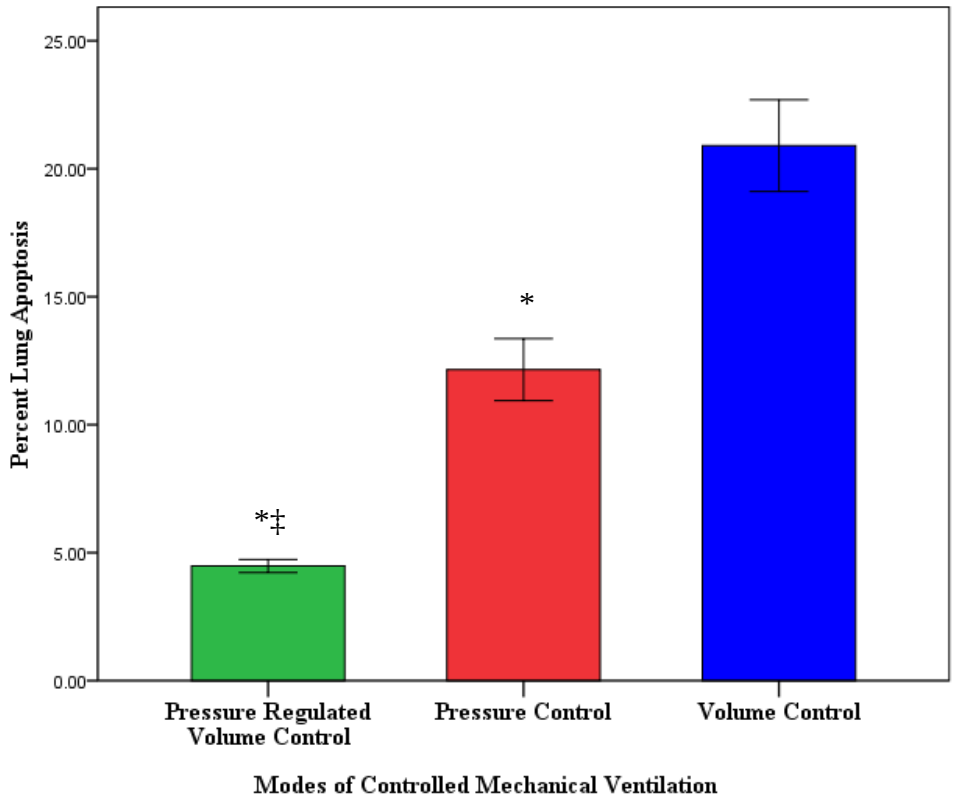


Figure 14. Percent lung apoptosis after 60 minutes of controlled mechanical ventilation during hemorrhagic shock
Data are presented as mean ± SEM, n = 6.
*P < 0.05 in comparison with volume control.
‡P < 0.05 in comparison with pressure control.

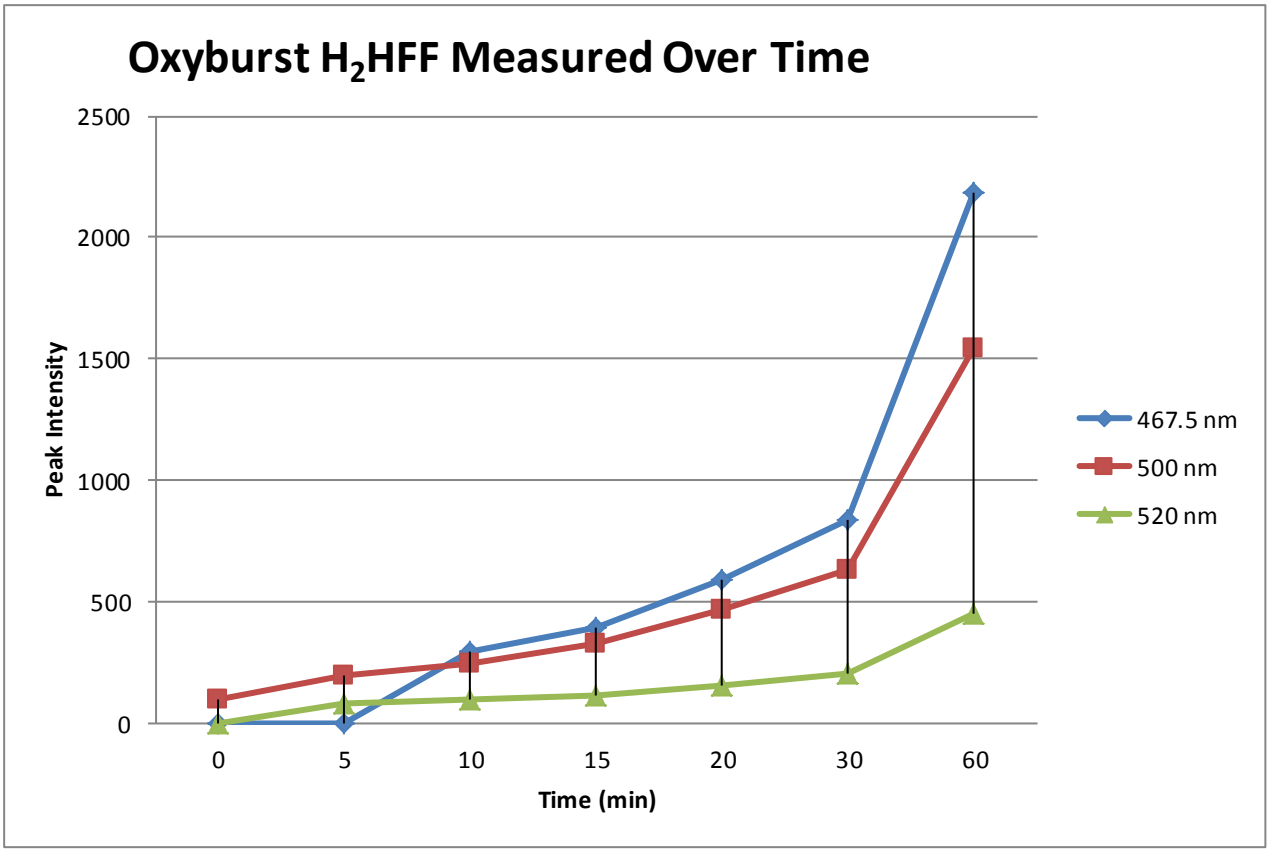
Summary of findings related to AIM 3: Following 60 minutes controlled mechanical ventilation with FIO_2 = 40% during hemorrhagic shock, we observed that the greatest lung hydrogen peroxide production and percent apoptosis was in the volume control group, followed by the pressure control group. The lung damage in the pressure-regulated volume control group was the least with the lowest hydrogen peroxide production and percent apoptosis.

Additional Funds AIM: To determine a biomarker of oxidative stress using rat blood before, during, and following hemorrhagic shock.

Research Objective 1: To investigate three biomarkers of oxidative stress in blood before, during and following hemorrhagic shock.

Three assay reagents were purchased from Molecular Probes to test this research objective. The first dye was called OxyBURST H_2HFF Green BSA. This is a sensitive fluorogenic reagent for detecting extracellular release of superoxide. The second dye was called dichlorodihydrofluorescein diacetate (H_2DCFDA, Fc OxyBURST), also known as dichlorofluorescein diacetate, was used to detect intracellular hydrogen peroxide, a precursor of the hydroxyl radical. The third dye was called MitoSOX Red, which is used to determine mitochondrial superoxide production. It is well established that mitochondria are a major intracellular source of superoxide.

Experiments were conducted to determine the extracellular release of superoxide before, during and after hemorrhagic shock in a rat model. The first biomarker OxyBURST H_2HFF was added to rat serum after each experimental period and incubated for 10 minutes. Using a spectrophotometer, the appearance of extracellular ROS was emitted at 528 nm when the dye was excited at 480 nm. The increased rate of fluorescein was assumed proportional to the concentration of extracellular superoxide. Twelve experiments were conducted in which the concentrations of OxyBURST H_2HFF varied from 100 to 400 µL in various volumes of serum (50 µL to 500 µL). With OxyBURST H_2HFF at a concentration of 400 µL in 500 µL of blood, peaks intensity exhibited at 467.5 nm, 500 nm, and 520 nm. These samples increased over time from 2 to 2250 peak intensity. We discovered that it required 60 minutes in order to detect the maximal peak intensity at those wavelengths (see figure below). The peak intensity was not significantly different from any time of experimental periods. In addition, new peaks were found in the lower fluorescent spectrum (300 nm) that ranged in the 10^4. After speaking with the Molecular Probes scientific advisor, they said that the spectrophotometer we used was not adequate to measure this photoprobe.

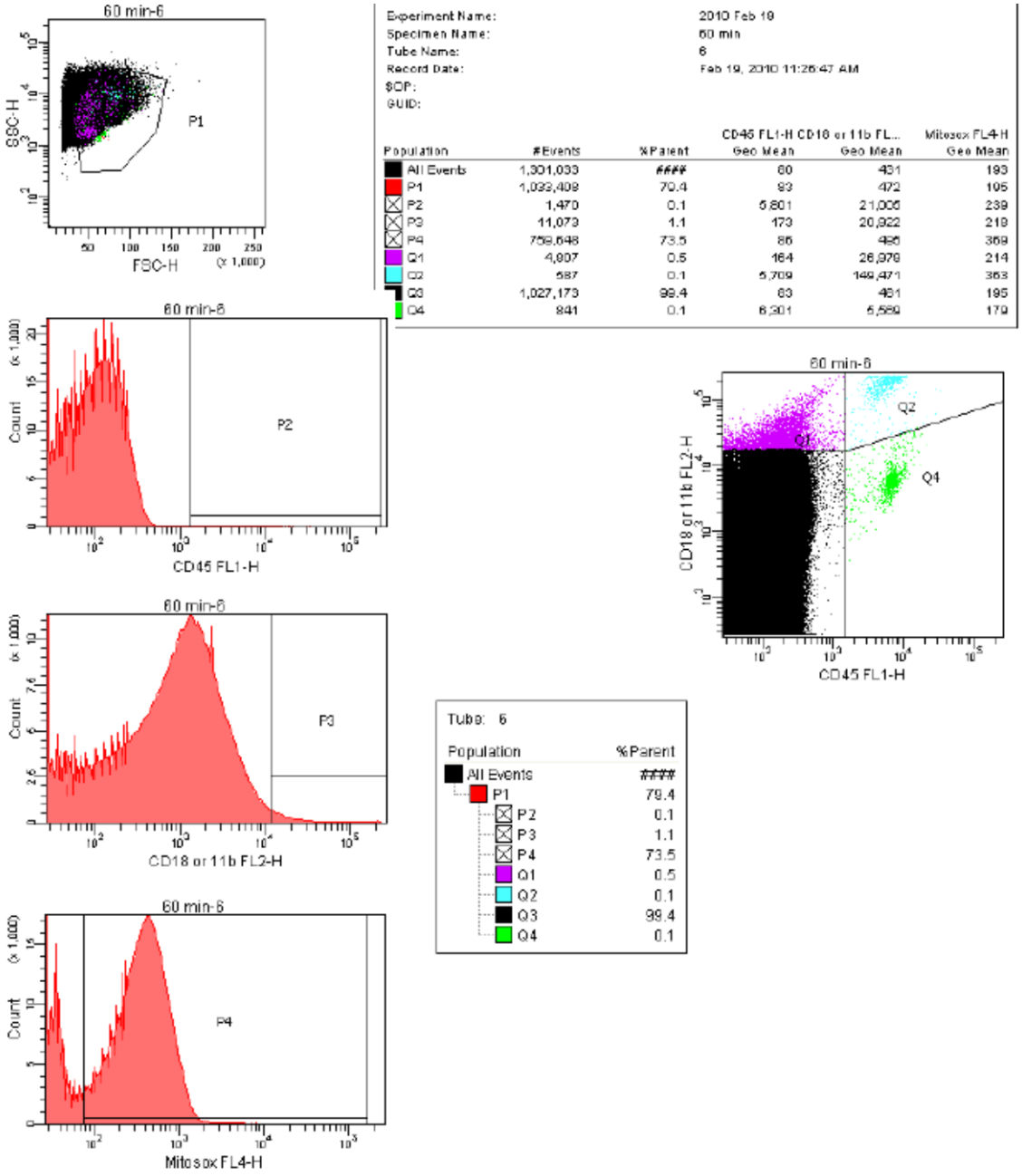


The second biomarker Fc OxyBURST was used to measure intracellular hydrogen peroxide at baseline, shock and 60 minutes after treatment. Fc OxyBURST was added to diluted blood and the concentration ranged from 25 µL to 75 µL. The mixture of blood and Fc OxyBURST were incubated for 10 minutes. Intracellular hydrogen peroxide was determined by fluorescent intensity using flow cytometry. With the use of monoclonal antibodies (Cluster of differentiation (CD)45, CD18, and CD11b), we were able to detect the differences in hydrogen peroxide production in these cell types. However, there were no differences in hydrogen peroxide production during these three time periods.

Experimental Time Period	CD45	CD18	CD11b
Control	1261 ± 399	319 ± 41	198 ± 32
Shock	1312 ± 241	406 ± 50	206 ± 51

Treatment	1238 ± 236	312 ± 39	189 ± 38

The third biomarker was MitoSOX Red, which was used to determine the production of superoxide in mitochondria. It is a fluorogenic dye that is oxidized by superoxide and exhibits red fluorescence. To isolate leukocytes, neutrophils and macrophages from red blood cells, monoclonal antibodies were used. With the addition of monoclonal antibodies, we were able to use whole blood and specifically identify different white blood cells that are producing superoxide. For example, we found that CD45 was sensitive in detecting leukocytes, CD18 for neutrophils, and CD11b for macrophages. When we combined the monoclonal antibodies (25 µL) with 1 mL of MitoSOX Red at the three experimental time periods, we were able to detect a difference in superoxide production in mitochondria using flow cytometry. Below is an example of the flow cytometry measurement of various cell types. Various fluorescent intensities are detected and differentiated based on cell type. For example, Q4 is the cell count for 179 neutrophils that have positive fluorescence for MitoSox Red.



<u>Research Objective 2:</u> To determine the effects of various FIO_2 concentrations on oxidative stress following hemorrhagic shock using three biomarkers.

In these experiments, we used anesthetized male Sprague-Dawley rats to measure superoxide in the mitochondria using the biomarker called MitoSOX Red at various oxygen concentrations. We chose MitoSox Red as our biomarker for oxidative stress based on the previous biomarker experiments mentioned above. We examined 12 rats at FIO_2 of 0.21, 0.40,

0.60, and 1.00 and measured MitoSox Red. Serial arterial blood samples were drawn at 0, 30, and 90 minutes of hemorrhagic shock. The biomarker MitoSox Red was added to blood sample with monoclonal antibodies of CD45, CD18, and CD11b. Following 10 minutes of incubation with MitoSox Red, blood samples were examined using fluorescent flow cytometry (BD LSRII) to determine fluorescent intensity.

The greatest amount of superoxide production in the mitochondrial was found in rats following hemorrhagic shock that received a FIO_2 at 0.21, 0.60, and 1.00. The least amount of oxidative stress was observed following administering oxygen at FIO_2 of 0.40. To determine the amount of oxidative stress in mitochondria specifically in neutrophils or macrophages, ratios were calculated. The table below summarizes the ratios of CD45/18 and CD45/11b. Decreased CD ratios indicate a reduction in mitochondrial superoxide.

Stage of the Experiment	CD18/45 ratio (all neutrophils)	CD11b/CD45 (macrophages)
Baseline	1.25 ± 0.29	1.39 ± 0.23
Hemorrhagic Shock	1.82 ± 0.36	1.55 ± 0.22
FIO_2 = 0.40	1.39 ± 0.10	1.40 ± 0.07

b. Relationship of current findings to previous findings:

AIM 1 AND AIM 2

The effects of various FIO_2 (0.21, 0.40, 0.60, and 1.00) and dopamine (10 µg/kg/min) on lung and diaphragm hydrogen peroxide and apoptosis following 60 minutes of hemorrhagic shock were investigated in our study. We observed that hydrogen peroxide and percent apoptosis in the lungs and diaphragm were the lowest when rats were administered 40% O_2. The degree of hydrogen peroxide production and apoptosis were similar in the animals breathing room air or 100% O_2, having the greatest amount. With the addition of dopamine with supplemental O_2, there

were significant decreases in lung and diaphragm hydrogen peroxide and apoptosis in all FIO_2 groups except in the FIO_2 at 0.40.

Lung injury can be caused by increased production of hydrogen peroxide during hemorrhagic shock, which is due to reduced oxygen delivery and cellular hypoxia (Souza, Poggetti, Fontes, & Birolini, 2000). However, there have been studies reporting that increasing oxygen supply by using a higher concentration of inspired oxygen may lead to hyperoxic induced lung damage. For example, Turrens and associates found that lung mitochondrial hydrogen peroxide production increased at FIO_2 greater than 0.60 (Turrens, Freeman, & Crapo, 1982). Similarly, other researchers reported lung injury accompanied by increased free radical formation during hyperoxia (Budzinska & Ilasz, 2008; Valenca Sdos et al., 2007). Our findings that the amount of lung hydrogen peroxide was influenced by the concentrations of inspired oxygen are consistent with these previous studies.

Our data indicated that a FIO_2 of 0.40 was most beneficial in minimizing lung hydrogen peroxide production following hemorrhagic shock. The probable cause of the reduced production of hydrogen peroxide with the administration of 0.40 relates to the enhanced antioxidant functions of the cells. Vento and colleagues observed that an FIO_2 of 0.30 increased glutathione free radical scavenging in prenatal neonates (Vento et al., 2009). In addition, Lee et al. found that mice breathing 40% oxygen had elevated tissue levels of vitamin E and C, known antioxidants (Lee et al., 2005). Thus, our data further supported the findings from these studies.

In our study, adding dopamine with different FIO_2s resulted in significant reduction in lung hydrogen peroxide at all FIO_2s except 0.40. These results suggest that dopamine scavenges free radicals in the lung tissue. This was consistent with the findings reported by Gero et al. who found that activating dopamine receptors were cytoprotective against H_2O_2 induced lung injury

(Gero et al., 2007). In addition, dopamine can enhance tissue O_2 perfusion, increase cardiac contractility, systemic pressure and heart rate during hemorrhagic shock (Holmes & Walley, 2003).

Apoptosis, or programmed cell death has been associated with hemorrhagic shock (Moran et al., 2008). Studies have shown that hemorrhagic shock can induce up-regulation of lung genes that are responsible for apoptosis (Shih, Wei, & Lee, 2005). High concentrations of FIO_2 may cause free radicals (i.e., superoxide) and induce mediated apoptosis (Buccellato, Tso, Akinci, Chandel, & Budinger, 2004). Releasing of cytochrome c from the mitochondria caused by increased free radicals leads to cell death (Pagano, Donati, Metrailler, & Barazzone Argiroffo, 2004). In our study, administering FIO_2 of 0.21 and 1.00 following 60 minutes of hemorrhagic shock resulted in the greatest percent lung apoptosis. This was consistent with the findings of lung hydrogen peroxide production with FIO_2 at 0.21 and 1.00. Following hemorrhagic shock, a FIO_2 of 0.40 produced the least amount of lung H_2O_2 and apoptosis.

Activated leukocytes are a source of free radicals during hemorrhagic shock (Childs et al., 2002). Dopamine has been shown to reduce polymorphonuclear leukocyte superoxide production (Yamazaki, Matsuoka, Yasui, Komiyama, & Akabane, 1989). In our study, dopamine reduced the percent lung apoptosis at three concentrations of inspired oxygen (0.21, 0.60, and 1.00) possibly as of a result of decreasing hydrogen peroxide. Our results were similar to the findings by Teramoto and associates who reported that lung apoptosis induced by hydrogen peroxide was in part attributable to production of reactive oxygen species (Teramoto et al., 1999).

The effect of free radicals on diaphragm muscle function has been well studied (Li et al., 2000; Supinski & Callahan, 2005). For example, free radicals decrease the release of calcium

from diaphragm muscle cells resulting in attenuated force generation, which could potentially lead to respiratory distress (Supinski & Callahan, 2005). Diaphragm reactive oxygen species are produced during re-oxygenation following hypoxia (Zuo & Clanton, 2005). However, free radicals could also be generated in the diaphragm during hyperoxia (Anzueto et al., 1994). This is supported by the findings in our study that administering of FIO_2s at 0.21 and 1.00 were accompanied by the greatest amount of diaphragm hydrogen peroxide. In contrast, administering FIO_2 of 0.40 resulted in the least amount of diaphragm hydrogen peroxide. The combination of dopamine with different FIO_2 except at 0.40 significantly decreased production of hydrogen peroxide in the diaphragm. This could be due to increased diaphragm blood flow induced by dopamine reported in one of our previous studies (J. D. Pierce, Clancy, Smith-Blair, & Kraft, 2002), which results in increased in oxygen delivery thus reduces formation of reactive oxygen species. The percent of diaphragm apoptosis at various FIO_2s were in consistent with changes in diaphragm hydrogen peroxide and percent lung apoptosis. The percent diaphragm apoptosis was highest at FIO_2s at 0.21 and 1.00 and lowest at 0.40. The addition of dopamine to various FIO_2 resulted in a significant reduction in the percent of apoptosis. This finding supports that dopamine is a free radical scavenger that decreases apoptosis in the diaphragm (J. D. Pierce, Goodyear-Bruch, Hall, Reed, & Clancy, 2008).

AIM 3:

We investigated the effects of three control modes of mechanical ventilation (volume control, pressure control, and pressure-regulated volume control) on lung damage measured by lung hydrogen peroxide production and apoptosis in a rat model following 60 minutes of hemorrhagic shock. Our data suggest that lung hydrogen peroxide and percent apoptosis were greatest in the volume control mode of mechanical ventilation, followed by pressure control. The

extent of lung hydrogen peroxide and apoptosis were lowest in the pressure-regulated volume control mode of ventilation. The differences in lung hydrogen peroxide and apoptosis during the three modes of controlled mechanical ventilation may not be attributable to differences in tissue oxygen delivery or the gas exchange levels as comparable hemodynamics and arterial blood values were relatively maintained in the three groups.

The action of mechanical ventilation can cause damage to the lungs directly, which is called ventilator induced lung injury. Research has shown that ventilator induced lung injury can lead to alveolar apoptosis (Le et al., 2008; Syrkina, Jafari, Hales, & Quinn, 2008).Barotrauma and volutrauma are two terms to describe macroscopic injuries caused by mechanical ventilation, which are related to alveolar over distension from high inspiratory pressures (Marcy, 1993) and excessive volume (Gattinoni, Protti, Caironi, & Carlesso, 2010), respectively. In our study, we did not find significant variations between the peak inspiratory pressures, tidal volumes, or inspiratory and expiratory intrathoracic pressures among the animals administered volume control, pressure control, or pressure-regulated volume control mechanical ventilation. Therefore, these routinely used respiratory parameters did not reflect the significant differences in hydrogen peroxide and apoptosis across the three modes of controlled mechanical ventilation.

The differences in lung hydrogen peroxide and apoptosis could be attributed to differences in the mechanics of gas delivery across the three modes of controlled mechanical ventilation. For example, inspiratory pressure with pressure-regulated volume control is continuously adjusted for variations in lung compliance, thereby, maintaining the preset tidal and minute volume (Burns, 2008; L. Pierce, 2007). In contrast, pressure control is to deliver breaths at a constant preset pressure with decelerating flow during a preset time and frequency. With volume control, a preset tidal volume is delivered at a constant flow during a preset time and

frequency (Kallet, Campbell, Alonso, Morabito, & Mackersie, 2000). Inspiratory decelerating flow patterns are utilized in both pressure control and pressure-regulated volume control. The least amount of hydrogen peroxide production and apoptosis found in the pressure-regulated volume control mode may relate to the continuous adaptation of inspiratory pressures to maintain the preset tidal and minute volumes. Decelerating inspiratory flow may improve oxygenation by early filling of alveoli and maintaining alveolar pressure longer. This leads to more homogenous gas distribution and better ventilation and perfusion matching, when compared to constant flow used in the volume control mode (Cheifetz, 2003; Dembinski et al., 2004; Unzueta, Casas, & Moral, 2007). Decelerating flow pattern also might have attenuated the shear stress on epithelial cells and alveolar cells, as compared to the constant flow pattern in the volume control mode. In our previous study, we found that lung hydrogen peroxide and apoptosis in hemorrhagic shock rats in the absence of mechanical ventilation on 40% O_2 were $0.5 \times 10^6 \pm 0.53$ and 2.38 ± 0.40, respectively (Mach, Thimmesch, Slusser, Clancy, & Pierce, 2010). These were comparable to the hydrogen peroxide production and lung apoptosis in the pressure-regulated volume control mechanical ventilation in our current study. Thus, it appears that mechanical ventilation with pressure-regulated volume control did not affect the extent of lung damage as measured by hydrogen peroxide and apoptosis during hemorrhagic shock.

ADDITIONAL FUNDS AIM:

In our study, we were able to measure oxidative stress caused by various fractional inspired oxygen (FIO_2) following 60 minutes of hemorrhagic shock using MitoSox Red. We found that the highest amount of mitochondrial superoxide production was among rats administered a FIO_2 at 0.21, 0.60, and 1.00. Administering a FIO_2 at 0.40 was associated with the lowest superoxide production in the mitochondria. These findings are consistent with the

results of generation of hydrogen peroxide in the lungs and diaphragm measured in AIM 1 and 2 in our current study. Our results indicate that MitoSox Red is a useful biomarker for measuring oxidative stress in the mitochondria, which is supported in previous studies that utilized MitoSox Red in oxidative stress studies (Mukhopadhyay, Rajesh, Hasko, et al., 2007; Mukhopadhyay, Rajesh, Yoshihiro, Hasko, & Pacher, 2007).

The findings of experiments with biomarker OxyBURST H_2HFF were not as expected. We were not able to detect significant differences in peak intensity from OxyBURST H_2HFF at different concentrations or at the various experimental periods (before, during, and after hemorrhagic shock). Other investigators have used OxyBURST H_2HFF to detect extracellular production of superoxide (Giambelluca & Gende, 2008; Zhang et al., 2007). However, in these experiments, the specific methods were not explicitly outlined, thus the protocol to use OxyBURST H_2HFF will need further investigation. Hence, we are not able at this time to make inclusive conclusions concerning OxyBURST H_2HFF as a biomarker for hemorrhagic shock.

The results of experiments using the biomarker called Fc OxyBURST were similar with those using OxyBURST H_2HFF. No significant differences in production of hydrogen peroxide were observed across three time periods of hemorrhagic shock (control, onset of hemorrhagic shock, and following 60 minutes of hemorrhagic shock). These findings were inconsistent with the results of studies using Fc OxyBURST in which they were able to detect increased hydrogen peroxide production in various cell types (Behndig, 2008; Wang et al., 2002).

c. Effect of problems or obstacles on the results:

One of the obstacles when we first started our experiments was the measurement of lung H_2O_2. We had previously performed laser scanning cytometry for H_2O_2 production using the diaphragm and had excellent results. However, when we first attempted to obtain H_2O_2

measurements in the lung, often the samples were too thick for accurate data. We worked with staff in the cytometry center and found that dissecting the lung tissue into thinner tissue samples assisted us with obtaining reliable H_2O_2 measurements. Over the next two years, we consistently acquire H_2O_2 data in lung tissue.

When we first started our mechanical ventilation studies during the administration of control modes of mechanical ventilation, the animals would often begin to take a few spontaneous breathes. That would cause our measurements of minute ventilation, respiratory rate and peak inspiratory pressures to vary. To resolve this problem, we began to check the animal every 10 minutes after we started mechanical ventilation for the level of anesthesia. We would pinch their toe nail bed and if we saw any increase in respiratory rate or movement, we would immediately administer a small amount of sodium pentobarbital intravenously. With this intervention, the animals no longer took any breathes spontaneously and our experimental parameters remained constant.

During the beginning of the granting period, we noticed some fluctuations in our apoptosis measurements. We discovered that older solutions of our apoptotic dyes appeared to make our nuclear dye staining quench. To resolve this issue, we found that that we needed to make new ethidium bromide and acridine orange dye solutions every two months to maintain consistent apoptosis measurements. With this intervention, we had no more unexpected alterations in our experimental results.

Our biggest obstacles came during our experiments related to the biomarker experiments. We began testing three dyes (OxyBURST H_2HFF, Fc OxyBURST, and MitoSox Red). We did not know the amount of monoclonal antibodies or biomarker dye to use. Thus, we attempted using various concentrations of the biomarker dyes with a known amount of H_2O_2. Once we

found the correct amount of the biomarker dye to utilize, we then began altering the amounts of the monoclonal antibodies. Using a dose response curve, we found the minimal amount of antibodies to use with each sample.

Another obstacle that we had with the OxyBURST H_2HFF dye related to the inconsistency we observed in the excitation peak that was found. Often the control value was greater than the hemorrhagic shock period value, which could not be corrected. We worked closely with Molecular Probes (manufacturer of the biomarker) to conduct a series of experiments that varied the amount of serum we used or the amount of time the dye stayed in the sample. We discovered that the biomarker dye had to be in the serum for almost one hour. We also found that we had to place the biomarker immediately in the sample before we began to centrifuge the sample to obtain the serum. It appeared that the period of centrifugation could increase the amount of reactive oxygen species that were produced during processing of the sample. With all these alterations, we then found a new and larger excitation peak in a lower fluorescent range in which the biomarker should not excite. After discussing with the Molecular Probes scientific advisor about our findings, they said that the instrument we used to measure this biomarker probably would not work with our spectrophotometer. Thus, in the future they suggested we needed to conduct a new set of experiments using a different instrument called a spectrofluorometer.

d. Limitations:

One of the largest limitations of our studies was that our findings from these animal studies are not directly generalizable to humans. Studies could be conducted in humans that experience hemorrhagic shock but first the biomarker (MitoSox Red) needs to be tested in patients to determine if it can measure the reactive oxygen species changes with treatment (lower levels of oxygen or administration of dopamine).

Another limitation was the use of the flow cytometry instrument. We had to estimate our different experimental periods (control, hemorrhagic shock and treatment) and schedule the time with the staff and an opening when the flow cytometry was not in use. If we did not estimate the time correctly, we would either have to wait for our sample analysis, which could alter our data or discard the sample because the instrument was not free to test our samples. With Dr. Slusser (Director of the Cytometry Center), we were able to manage both the times and the instrumentation. Unfortunately, at the end of the grant, Dr. Slusser obtained employment with another company and we temporarily lost our scientific expert to assist us with our biomarker measurements.

A limitation of our study was the short time period of mechanical ventilation (60 min), which may have prevented us from observing any macroscopic changes in pulmonary mechanics. Another limitation was that we did not administer any fluid resuscitation to correct the hemorrhagic shock during mechanical ventilation. This allowed us to examine the effects of controlled mechanical ventilation in the absence of changes in free radicals associated with fluid resuscitation. Testing only a controlled mode of mechanical ventilation was a limitation to our study. Most mechanical ventilations offer a mixed mode of mechanical ventilation that includes both controlled and assisted modes of ventilation. When a patient is recovering from a hemorrhagic shock, offering the patient the opportunity to assist the mechanical ventilator would be optimal in weaning from the mechanical ventilation.

Adding the reactive oxygen species biomarker after each experimental period to the blood sample is a limitation. The ideal method would be to administer the biomarker at the beginning of the experiment to the animal and then obtain a blood sample for each experimental

period. Unfortunately, these biomarkers cannot be given intravenously to the animal and then be extracted via a blood specimen.

e. Conclusion:

We have four major conclusions from our grant. First, when an animal experiences hemorrhagic shock, the optimal oxygen that should be used is a FIO_2 of 0.40 (40%). If higher or lower concentrations of oxygen are used then free radical damage is produced from the extra oxygen molecules. The second major finding is that if a higher oxygen concentration is needed after a hemorrhagic event to prevent hypoxia, dopamine (10 μg/kg/min) serves as a free radical scavenger and prevents lung and diaphragm damage. The third major finding relates to the optimal control mode of mechanical ventilation to use following hemorrhagic shock. Pressure regulated volume control produced the least amount of reactive oxygen species and apoptosis when compared to volume control or pressure control ventilation. The fourth major finding is a biomarker to measure reactive oxygen species in the blood. We found that MitoSox Red as potential biomarker to use to measure superoxide in mitochondria of neutrophils and macrophages following hemorrhagic shock.

The current standard of practice is to apply oxygen to a military hemorrhagic shock patient at the highest concentration possible (usually FIO_2 of 100%). However, too much oxygen can produce free radicals and damage tissues and cells and actually cause increased organ damage. Therefore, our study has investigated the optimal concentration of oxygen to administer after a hemorrhagic event using an animal model. Our results indicate that too little or too much oxygen after hemorrhagic shock can produce diaphragm and lung injury. The optimal level of oxygen is a FIO_2 of 0.40, which provides sufficient oxygen to the tissues without producing too many reactive oxygen species that can lead to tissue damage.

Another question we investigated related to the use of a commonly used drug called dopamine to infuse as a scavenger for free radicals with oxygen administration in a hemorrhagic shock model. We found that if a higher concentration of oxygen is used with dopamine (10 µg/kg/min) following hemorrhagic shock, the animal had significantly reduced amounts of diaphragm and lung damage as indicated by hydrogen peroxide production and apoptosis. This has great military clinical nursing implications because it provides the data needed to support the usage of a scavenger drug during a hemorrhagic event. If one of our warfighters experiences hemorrhagic shock and needs a higher concentration of oxygen to maintain respiratory efforts, dopamine could be infused to prevent additional injury from the oxygen administration.

Our additional findings suggest that the optimal mechanical ventilation mode is a combination of pressure and volume control following hemorrhagic shock. In military field hospitals and most often in military hospitals nurses care for patients on a volume support modes that do not have this combined feature. Thus, the mode of mechanical ventilation that our warfighters are being administered could be causing additional lung and diaphragm damage. Future military nursing research in practice could be focused on measuring the amount of oxidative stress produced by the different modes of mechanical ventilation following hemorrhagic shock. Our research on the biomarkers indicated that MitoSox Red is a potential indicator of increased reactive oxygen species produced in the mitochondria of leukocytes. Using only 25 µL of whole blood with a monoclonal antibody could be a way for nurses to monitor free radical generation and prevent damage from excess production of harmful molecules.

Currently, there is no active intervention for non-compressible hemorrhage available to military or civilian medics and physicians; however, research of non-compressible hemorrhage control methods may offer solutions that could save lives. A systemic drug that could be given

intravenously to induce coagulation or a device that compresses or occludes the bleeding vessel are potential treatments of truncal hemorrhage that have promise.

The administration of too much oxygen may result in hyperoxia and increased free radical production, DNA damage, disease, and even death. Free radical production increases during hemorrhagic shock, and often times requires oxygen administration. In order to limit increases in free radicals such as hydrogen peroxide, administering lower fraction of inspired oxygen is necessary. Our data suggest that a FIO_2 at 0.40 is the most optimal when administering oxygen following HS. A significant increase in lung and diaphragm H_2O_2 and apoptosis occurs with room air (0.21) and the administration of FIO_2s at 0.60 and 1.00. With the addition of dopamine, these increases were prevented. Current practice is the administration of 100% oxygen, if this percent is necessary, dopamine administration will assist in minimizing free radical production thus, preventing cellular death, tissue damage, and disease. Mechanical ventilation is often used with HS patients and may result in lung injury and free radical production. This production and injury varies with different controlled modes of mechanical ventilation including volume control, pressure control, and pressure-regulated volume control. With regards to lung H_2O_2 and apoptosis measurements, our findings were that the optimal controlled mode of mechanical ventilation to use with 40% oxygen post hemorrhagic shock was pressure-regulated volume control, followed by pressure control and volume control.

Patients experiencing hemorrhagic shock produce excessive amounts of free radicals, specifically reactive oxygen species. Hence, obtaining blood specimens from hemorrhagic shock patients could provide a biomarker to determine the oxidative stress of resuscitation therapies. The additional funds received has allowed us to begin our investigation on blood biomarkers that

measure oxidative stress which could lead to new clinical therapeutic strategies for military personnel experiencing hemorrhagic shock.

The next step in the research process is two-fold. First, there needs to be a study conducted to begin translation of these findings to humans. This could be started with measurement of MitoSox Red in human blood and after a hemorrhagic event. A descriptive study could be conducted to determine the type and amount of oxygen administered to hemorrhagic shock patients and when in the process of care are initiated. The final step would be to give 40% oxygen or higher concentrations with dopamine and determine if superoxide is increased in the mitochondria. Other macroscopic physiologic measurements could be collected such as arterial blood gases, hemodynamics and oxygen saturation.

The second part would be to obtain funding to continue examining biomarkers like OxyBURST for potential ways of measuring generation of oxygen species. These types of biomarkers could be valuable to examine all types of military nursing interventions such as fluid resuscitation, suctioning, weaning from mechanical ventilation and drug administration. Guiding military clinical nursing using biomarkers would provide an evidence-based method in delivering care to our wounded warriors.

VI. SIGNIFICANCE OF STUDY OR PROJECT RESULTS TO MILITARY NURSING

Military nursing clinical practice: Currently, care for our wounded warriors experiencing hemorrhagic shock includes administering high concentrations of oxygen. Excessive molecules of oxygen in the body can form damaging free radicals that can cause severe organ damage to military personnel. Our studies have found that concentrations of oxygen at FIO_2s of 0.21, 0.60, and 1.00 caused increased lung and diaphragm damage from free radicals. However, using 40% of oxygen was optimal in supplying oxygen to tissue without damaging cells. Thus, military nursing clinical practice should begin with administering oxygen at 0.40 and if needed, a free radical scavenger such as dopamine should be given as a preventive intervention to assist our wounded warriors in a hemorrhagic shock event. In addition, we found that a mode of mechanical ventilation that provides pressure and volume also reduced free radical formation. Therefore, military nurses should encourage usage of modes of mechanical ventilation that ensure optimal tidal volume with minimal pressure for military personnel following HS.

Military nursing leadership: Military nursing leadership can use these data to indicate that many of the practices used in our current care for our wounded warriors is not evidenced based. Our nursing leaders could illustrate how military nursing research has now shown that the current standard of practice for oxygen administration for military personnel experiencing hemorrhagic shock should be further investigated to prevent additional injury to our patients. They could investigate the cost, tissue injury, and patient suffering that could be reduced with monitoring reactive oxygen species during hemorrhagic events and use of the optimal oxygen (FIO_2 = 0.40) with dopamine and/or with PRVC.

Military nursing management: Nurses in military facilities in charge of nursing management could utilize our finds to reinforce to nursing staff the need for evidenced based

practice and not just standard written protocols. Again, a study could be conducted by military nurse management to determine the amount of oxygen nurses administer to hemorrhagic shock patients during an emergency and the type of mechanical ventilation mode that is used. With these type of data, military nurse managers could begin to standardize protocols based on recent data that relates to hazards from oxygen administration.

Military nursing education: Our research data are the type of information that should be disseminated at Uniformed Services University, military hospitals and military trauma courses. This is the evidence needed to improve military patient outcomes by educating military nurses, corpsman, medics and other health care professionals about the damaging effects of reactive oxygen species generated by oxygen administration. The physiologic principles of oxygen supply and oxygen demand are demonstrated by our data and may introduce to military health personnel more of a cellular/molecular perspective to warfighter care.

Military nursing policy: Hemorrhagic shock results in a significant percent of mortality and the policy related to knowing the proper treatment is essential to prevent increased tissue damage and death. It has been a standard of practice in the military to administer 100% oxygen when a patient experiences hemorrhagic shock. According to our results, oxygen at this level produces a high amount of free radicals, as measured by H_2O_2 and thus lung and diaphragm apoptosis. With a high number of military patients with HS occurring during Operation Iraqi and Enduring Freedom, awareness that using a lower FIO_2 at 0.40 or administering dopamine with higher FIO_2s results in a low amount of H_2O_2 and apoptosis. This is especially important since wounded warriors spend a lot of time during transport by air or ambulance under the care of military nurses who would administer oxygen and/or dopamine.

VII. CHANGES IN CLINICAL PRACTICE, LEADERSHIP, MANAGEMENT, EDUCATION, POLICY, AND/OR MILITARY DOCTRINE THAT RESULTED FROM STUDY OR PROJECT

One of the highest priorities of the US military in care of our troops is the treatment of the injured, particularly those that experience hemorrhagic shock. During Operation Iraqi Freedom and Operation Enduring Freedom many of the battle injuries are to the torso where compression cannot be applied, major vessels are damaged and there is large blood loss. The management of hemorrhagic shock patients is essential for military nurses in clinical practice in theater or at large military hospital facilities. Following a hemorrhagic event there is reduced hemoglobin to transport oxygen in the blood thus the oxygen demand is increased. Both mechanical ventilation and hemorrhagic shock produce free radicals so finding the optimal concentration of oxygen with the ideal mode of mechanical ventilation is extremely useful for military clinical practice, leadership, management, education, and policy that will help keep the production low and therefore prevent tissue injury. The nature of our study relates to the essential characteristic of oxygen when used as a treatment for hemorrhagic shock. The overall scope of these experiments related to finding the optimal concentration of oxygen that could be used during hemorrhagic shock that was useful in treating the hypoxic physiologic state and at the same time not produce harmful free radicals.

Our studies have discovered **three key outcomes** extremely important as the foundation for military nursing and all of healthcare as it relates to the care of hemorrhagic shock patients. The first relates to administering oxygen at an FIO_2 of 0.40 instead of 0.60 or 1.00 when a military personnel first experience hemorrhagic shock. The second relates to using dopamine as a free radical scavenger when a higher level of oxygen is needed in a hemorrhagic shock patient. By administering dopamine, the military nurse can reduce excessive amounts of reactive oxygen

species produced when the patient needs a higher percentage of oxygen (60% or 100%) thus reducing additional tissue damage. This intervention would provide adequate oxygen supply without producing too many harmful free radicals. In addition, in the field or battalion aid station, military personnel breathing room air ($FIO_2 = 0.21$) would have inadequate oxygen supply for the demand of the body since the hemoglobin concentration is reduced. Thus, oxidative stress would occur leading to tissue damage. Our data suggests that this could be limited with the use of dopamine intravenously at 10 µg/kg/min. This intervention is so important in reducing acute lung injury and strengthening diaphragmatic function. The third outcome relates to finding the optimal mode of control mechanical ventilation to use on a hemorrhagic shock patient. Our study found that when comparing volume controlled (VC), pressure controlled (PC), and pressure-regulated volume controlled (PRVC) mechanical ventilation, PRVC was the most optimal when using a FIO_2 at 0.40 in relation to lung H_2O_2 concentration and apoptosis. Using this mechanical ventilation mode in military hospitals may help prevent shear lung injury and reduce the number of days required on mechanical ventilation.

Biomarker research and practice is becoming increasingly important for military health care. Although we could not recommend two of the biomarkers we tested, we did find that MitoSox Red is a potential biomarker to determine superoxide production in leukocytes. This means that with as little as 25 µL of blood and a monoclonal antibody, we could determine a level of free radical production in mitochondria. With this biomarker, we could then monitor our military nursing interventions such as oxygen or drug administration to determine if they are decreasing or actually increasing the free radical production. This would be a truly evidence based practice method to determine on a cellular and molecular basis if nursing inventions are assisting or actually producing further injury to our wounded warriors.

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IX. SUMMARY OF DISSEMINATION

Type of Dissemination	Citation	Date and Source of Approval for Public Release
Publications	Cusimano, E., Knight, A., Slusser, J., Clancy, R., & Pierce, J. (2009). Mitochondria: the hemi of the cell. *Advanced Emergency Nursing Journal*, *31*(1), 46-54.	Date: 31 MAR 2009 Source: TSNRP Executive Director - Deborah J Kenny, PhD, RN, LTC, AN, USA
	Mach, B., Thimmesch, A., Slusser, J., Clancy, R., & Pierce, J. (2010). The effects of increased inspired oxygen with and without dopamine on lung and diaphragm hydrogen peroxide and apoptosis following hemorrhagic shock. *Journal of Pre-Clinical and Clinical Research, 4*(1), *005-010.*	Date: 30 OCT 2009 Source: TSNRP Executive Director - Marla J De Jong, Col, USAF, NC, PhD
	Mach, B., Knight, A., Orr, J., Slusser, J., & Piece, J. (2010). Flow cytometry and laser scanning cytometry, a comparison of techniques. *Journal of Clinical Monitoring and Computing*, *24*, 251-259.	Date: 31 MAR 2009 Source: TSNRP Executive Director - Deborah J Kenny, PhD, RN, LTC, AN, USA
	Knight, A., Fry, L., Clancy, R., & Pierce, J. (2011). Understanding the effects of oxygen administration in hemorrhagic shock. *Nursing in Critical Care*, *16*(1), 28-34.	Date: 29 MAY 2009 Source: TSNRP Executive Director - Deborah J Kenny, PhD, RN, LTC, AN, USA
	Mach, B., Knight, A., Pierce, T., & Pierce, J. (2011). Consequences of hyperoxia and the toxicity of oxygen in the lung. *Nursing Research and Practice*, *2011*(article ID 260482), 1-7.	Date: 3 OCT 2011 Source: TSNRP Executive Director – John P Maye, CRNA, PhD, CAPT, NC, USN
Publications in Review	Thimmesch, A., Shen, Q., Clancy, R., & Pierce, J. (2011). Effects of three controlled mechanical ventilation modes on rat lung hydrogen peroxide and apoptosis during hemorrhagic shock. Intensive Care Medicine, (in review).	Date: 18 OCT 2011 Source: TSNRP Executive Director – John P Maye, CRNA, PhD, CAPT, NC, USN

Published Abstracts	Pierce, J., Mach, W., Knight, A., & Pierce, J. T. (2009). Lung and diaphragm hydrogen peroxide and apoptosis measurements following hemorrhagic shock and different fraction of inspired oxygen concentrations and dopamine. *Critical Care Medicine, 37* (12 suppl), A171.	Date: 9 SEP 2009 Source: TSNRP Executive Director - Marla J De Jong, Col, USAF, NC, PhD
	Pierce, J, Thimmesch, A., Walsh, C., & Clancy, R. Effects of controlled modes of mechanical ventilation following hemorrhagic shock on lung hydrogen peroxide and apoptosis. *Visions: Critical Care Nursing*, Overland Park, KS, 2011.	Date: 24 JUN 2010 Source: TSNRP Executive Director - Marla J De Jong, Col, USAF, NC, PhD
Podium Presentations	Fry, L., Knight, A., Clancy, R., & Pierce, J. Lung and diaphragm hydrogen peroxide and apoptosis following the administration of various fractional inspired oxygen concentrations post- hemorrhagic shock. 5th Annual Symposium in Intellectual Pursuit in Undergraduate Nursing, Kansas City, MO, 6 NOV 2008.	Date: 22 SEP 2008 Source: TSNRP Executive Director - Deborah J Kenny, PhD, RN, LTC, AN, USA
	Pierce, J. Treatment of diaphragm and lung apoptosis following hemorrhagic shock. University of Kansas Seminar in Physiology, Kansas City, KS, 19 OCT 2009.	Date: 23 OCT 2009 Source: TSNRP Executive Director - Marla J De Jong, Col, USAF, NC, PhD

	Knowles, E., Thimmesch, A., Qiuhua, S., Clancy, R., & Pierce, J. Varying modes of mechanical ventilation and lung injury following hemorrhagic shock. Seventh Symposium on Intellectual Pursuit in Undergraduate Nursing, Kansas City, MO, 17 NOV 2011.	Date: 18 OCT 2011 Source: TSNRP Executive Director – John P Maye, CRNA, PhD, CAPT, NC, USN
Poster Presentations	Pierce, J., Mach, W., Knight, A., & Pierce, J. T. (2009). Lung and diaphragm hydrogen peroxide and apoptosis measurements following hemorrhagic shock and different fraction of inspired oxygen concentrations and dopamine. *Critical Care Medicine,* Miami, FL JAN 2010. Pierce, J, Thimmesch, A., Walsh, C., & Clancy, R. Effects of controlled modes of mechanical ventilation following hemorrhagic shock on lung hydrogen peroxide and apoptosis. *Visions: Critical Care Nursing Conference*, Overland Park, KS, 03 MAR 2011.	Date: 9 SEP 2009 Source: TSNRP Executive Director - Marla J De Jong, Col, USAF, NC, PhD Date: 24 JUN 2010 Source: TSNRP Executive Director - Marla J De Jong, Col, USAF, NC, PhD
Media Reports		
Other		

X. REPORTABLE OUTCOMES

Reportable Outcome	Detailed Description
Applied for Patent	none
Issued a Patent	none
Developed a cell line	none
Developed a tissue or serum repository	none
Developed a data registry	none

XI. RECRUITMENT AND RETENTION TABLE

Recruitment and Retention Aspect	Number
Animals Projected in Grant Application	190
Animals Purchased	190
Model Development Animals	0
Research Animals	190
Animals With Complete Data	180
Animals with Incomplete Data	10

XII. FINAL BUDGET REPORT

A reallocation of funds was requested to correct the deficit that was present in the personnel category. Upon acceptance of reallocation request, the monies from the other expenses and supplies categories were transferred to the personnel category. When this occurred, the deficits were corrected. The remaining funds were used to complete and disseminate the findings.

Additional funds were requested to investigate biomarkers of oxidative stress using fluorescent dyes in the blood following hemorrhagic shock and administration of different FIO_2s. Upon the approval of $90,000, the research was completed.